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CICATRIZATION OF WOUNDS.

I. The Relation between the Size of a Wound and the Rate of Its Cicatrization.

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In the course of experiments made in 1908 at The Rockefeller Institute certain relations existing between the size of a wound and the rate of cicatrization were studied. The experiments showed that the rate of repair was greater at the beginning than at the end of cicatrization, and depended not on the age of the wound but on its size, being directly proportional to it.[1] The skin of mammals seemed therefore to regenerate according to a law similar to that found by Spallanzani for salamanders. The experiments made lately by Spain and Loeb showed that in the guinea pig the same relation existed between the size of a wound and the rate of repair.[2] The object of the following experiments was to find a technique by which the size of a wound could be measured accurately, to ascertain whether the curve representing the cicatrization was geometric in form, and to study the relations between the size of a wound and the velocity of repair, as well as the relative importance of the processes of contraction and epidermization.

EXPERIMENTAL.

The experiments were made in the following manner. In the sternal region or in the anterior abdominal region of anesthetized guinea pigs and cats, wounds were obtained by resection of a strip of skin geometric in form. In order that the edges of the cicatrix

[1] Carrel, A., *J. Am. Med. Assn.*, 1910, lv, 2148.

[2] Spain, K. C., and Loeb, L., *J. Exp. Med.*, 1916, xxiii, 107.

might be seen distinctly, animals with a black skin were used, or the edges of the wound made on white animals were tatooed with India ink. The skin of the cat and, as had been previously noted, of the dog not being adherent to the aponeurosis, errors occurred in the measurement, if, in consecutive observations, the animal was not placed in an identical position. The guinea pig was generally employed because the skin of the abdominal wall of this animal is more adherent to the aponeurosis than that of the cat or dog. In human beings wounds of regular shape were selected, located on patients confined to bed. When both the wound and the cicatrix were to be studied, cases were chosen in which the outer edge was well colored and easily discernible from the surrounding skin. Observations were also made on the healing wounds of soldiers.

During the period of observation the wounds were kept by means of antiseptic and aseptic dressings in as constant a bacteriological condition as possible. Every day films of the secretions, taken in different parts of the wounds, were examined. When the films contained bacteria the wound and the surrounding skin were cleaned with a cotton sponge and neutral sodium oleate. Then, for a few hours, 0.5 per cent of Dakin's hypochlorite or 1 per cent paratoluene sodium sulphochloramide was instilled on the wound. As soon as the bacteria had disappeared, aseptic dressings or compresses moistened with 0.2 per cent paratoluene sodium sulphochloramide were applied. The wound remained aseptic for several days. If bacteria appeared again on the granulating surface, chemical sterilization was used.

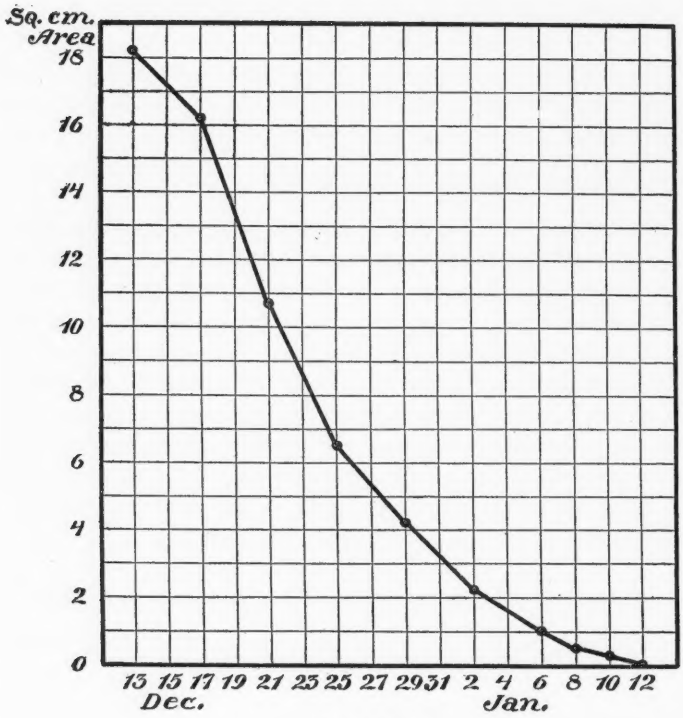
The measurements of the wound were taken at regular intervals, as a rule every 4 days. The procedure was as follows: After the surface was thoroughly dried by antiseptic gauze or by filter paper, a sheet of thin, transparent cellophane was applied with a sponge directly over the granulations and the epithelial edge. The inner edge of the epithelium could then be clearly discerned, and was even felt under the point of the wax pencil by means of which it was drawn on the cellophane. The outer edge of the pencil mark corresponded to the line of union between the epithelium and the granulating surface. Two drawings were made at each examination. The outer edge of the cicatrix was also outlined. The drawings obtained on

cellophane were then reproduced in ink on a sheet of paper. This second drawing was used for the measurements. By means of a planimeter the area of the wound (S) and the area of the wound and the cicatrix ($S + C$) were estimated in square centimeters. The daily rate (R) of cicatrization was obtained by dividing the differences of two consecutive surfaces by the time which had elapsed between the two observations. The value of the rate of cicatrization in its relation to the area was obtained by the formula $\frac{S}{V}$.

Through the values of S and R a curve was established in which the time was carried in abscissæ, and the area in ordinates. The tracing representing the variations of the surface located within the outer edge of the cicatrix was drawn above the curve of the surface of the wound. The interval between both curves represented the area of the cicatrix in square centimeters. Thus it was possible to ascertain daily, in square centimeters, the size of the wound, the size of the cicatrix, and the velocity of the process of repair. By means of this technique, the relations which exist between the size of a wound and the rate of cicatrization, and the respective value of contraction and of epidermization in the process of repair, were studied.

The Relation between the Size of a Wound and the Velocity of Repair.

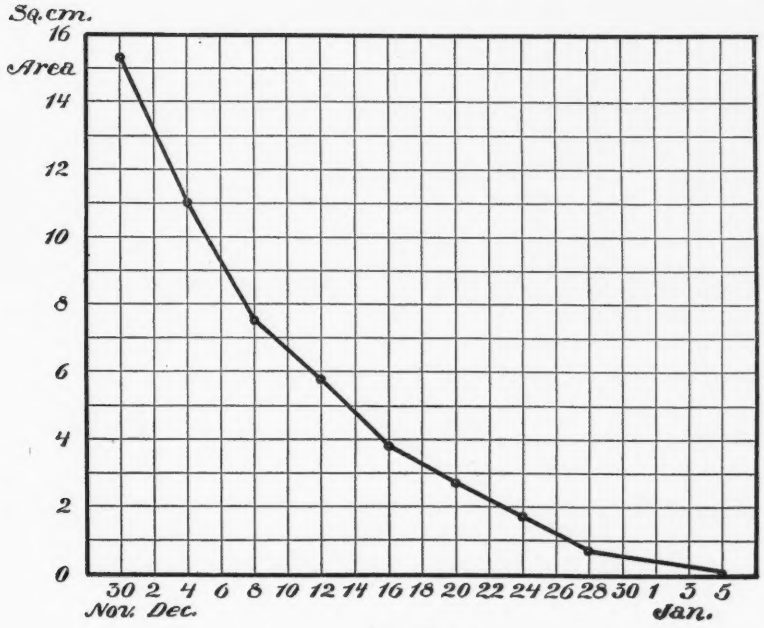
The observations were made on men and on guinea pigs. The wounds were in a condition of slight infection and healed both by contraction and by epidermization.



TEXT-FIG. 1.

Experiment 1.—Patient 221, age 27 years (Text-fig. 1). Old wound of the left foot; aseptic. Normal cicatrization.

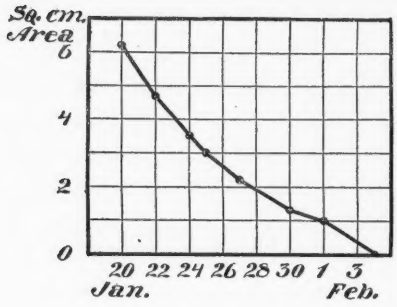
	Dec. 13	17	21	25	29	Jan. 2	6	8	10	12
S.....	18.2	16.2	10.7	6.5	4.2	2.2	1.0	0.5	0.3	0
R.....		0.5	1.37	1.05	0.55	0.5	0.3	0.25	0.1	0.15
$\frac{S}{R}$.....	36.0	11.8	10.1	11.8	8.4	7.3	3.0	5.0	2.0	



TEXT-FIG. 2.

Experiment 2.—Patient 217, age 36 years (Text-fig. 2). Wound of the left iliac region; slightly infected. The appearance of the curve is normal although the rate of repair was slow.

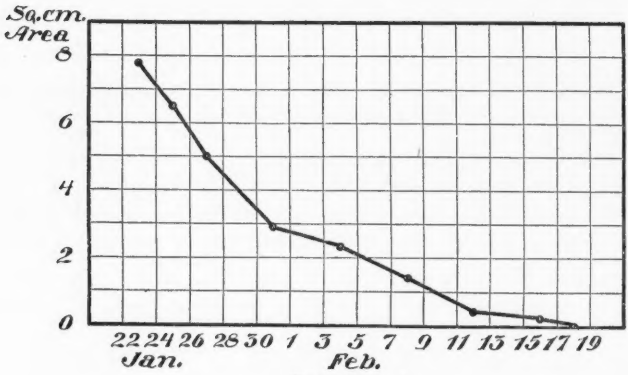
	Nov. 30	Dec. 4	8	12	16	20	24	28	Jan.
S.....	15.3	11.0	7.5	5.8	3.8	2.7	1.7	0.7	0
R.....		1.07	0.87	0.42	0.5	0.17	0.25	0.25	0.1
$\frac{S}{R}$.....	14.3	12.6	17.8	11.6	22.0	10.8	6.8	7.0	



TEXT-FIG. 3.

Experiment 3.—Patient 354, age 40 years (Text-fig. 3). Old wound on a stump; slightly infected. Normal cicatrization. The irregularity of the curve on Feb. 1 was due to the dressing.

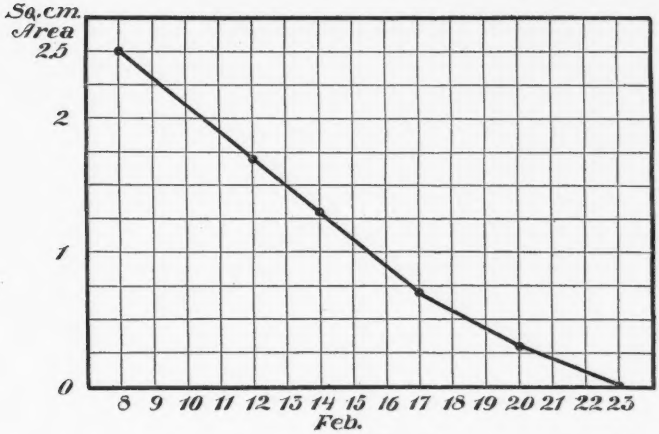
	Jan. 20	22	24	25	27	30	Feb. 1	4
S.....	6.2	4.7	3.5	3.0	2.2	1.3	1.0	0
R.....		0.75	0.60	0.50	0.40	0.3	0.15	0.33



TEXT-FIG. 4.

Experiment 4.—Patient 330, age 32 years (Text-fig. 4). Wound following the amputation of the index finger and of a part of the metacarpus; slightly infected. From Jan. 31 the rate of cicatrization decreased on account of infection.

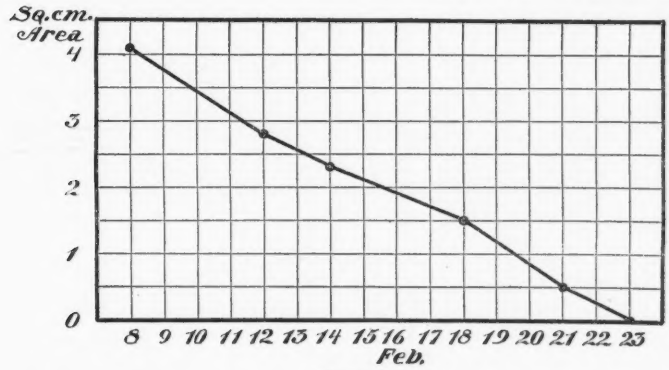
	Jan. 23	25	27	31	Feb. 4	8	12	16	18
S.....	7.8	6.5	5.0	2.9	2.3	1.4	0.4	0.2	0
R.....		0.65	0.72	0.52	0.15	0.22	0.25	0.05	0



TEXT-FIG. 5.

Experiment 5.—Guinea Pig 1 (Text-fig. 5). Wound of the anterior abdominal wall; slightly infected. Normal cicatrization.

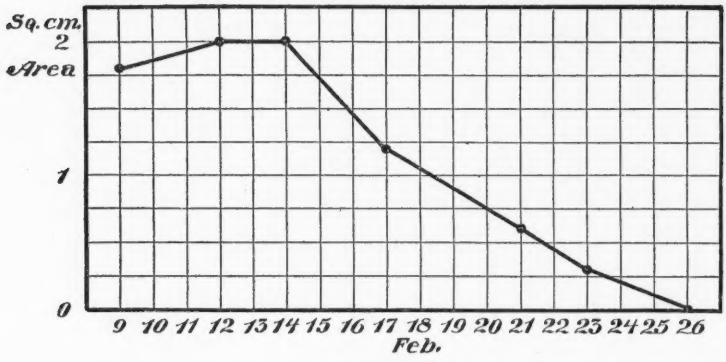
	Feb. 8	12	14	17	20	23
S.....	2.5	1.7	1.3	0.7	0.3	0
R.....		0.8	0.2	0.2	0.15	



TEXT-FIG. 6.

Experiment 6.—Guinea Pig 2 (Text-fig. 6). Wound of the anterior abdominal wall. The curve is nearly regular in spite of a slight infection. The slackening of the rate of cicatrization on Feb. 18 was due to a more marked condition of infection.

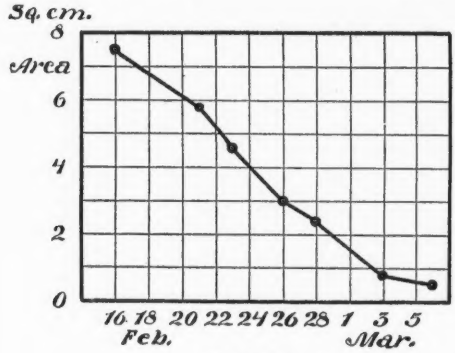
	Feb. 8	12	14	18	21	23
S.....	4.1	2.8	2.3	1.5	0.5	0
R.....		0.32	0.25	0.20	0.3	



TEXT-FIG. 7.

Experiment 7.—Guinea Pig 3 (Text-fig. 7). Wound of the anterior abdominal wall; slightly infected. The horizontal part of the curve from Feb. 9 to 14 represents the period normally preceding the beginning of contraction.

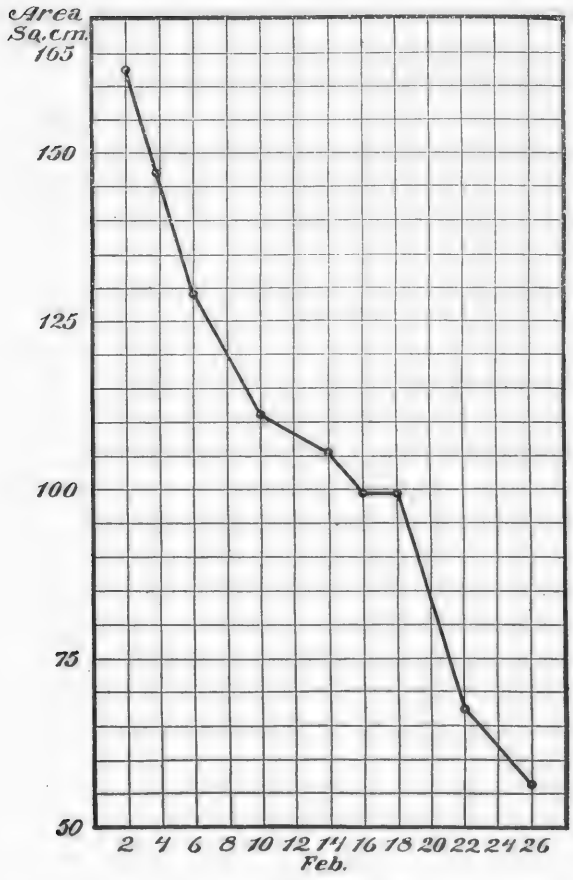
	Feb. 9	12	14	17	21	23	26
S.....	1.8	2.0	2.0	1.2	0.6	0.3	0
R.....				0.26	0.15	0.15	0.1



TEXT-FIG. 8.

Experiment 8.—Guinea Pig 4 (Text-fig. 8). Wound of the anterior abdominal wall. The slow rate of cicatrization from Feb. 16 to 21 was due to the period preceding the beginning of contraction.

	Feb. 16	21	23	26	28	Mar. 3	6
S.....	7.5	5.8	4.6	3.0	2.4	0.8	0.5
R.....		0.34	0.55	0.36	0.56	0.3	0.4



TEXT-FIG. 9.

Experiment 9.—Patient 360, age 21 years (Text-fig. 9). Wound of the abdominal wall. The horizontal part of the curve from Feb. 16 to 18 represents a period of infection. As soon as the wound was sterilized chemically, the curve descended abruptly.

	Feb. 2	6	10	14	16	18	22	26
S.....	162.5	129.4	111.0	105.5	99.5	99.5	67.5	57.0
R.....		8.25	4.6	1.3	3.0	0	8.0	2.6

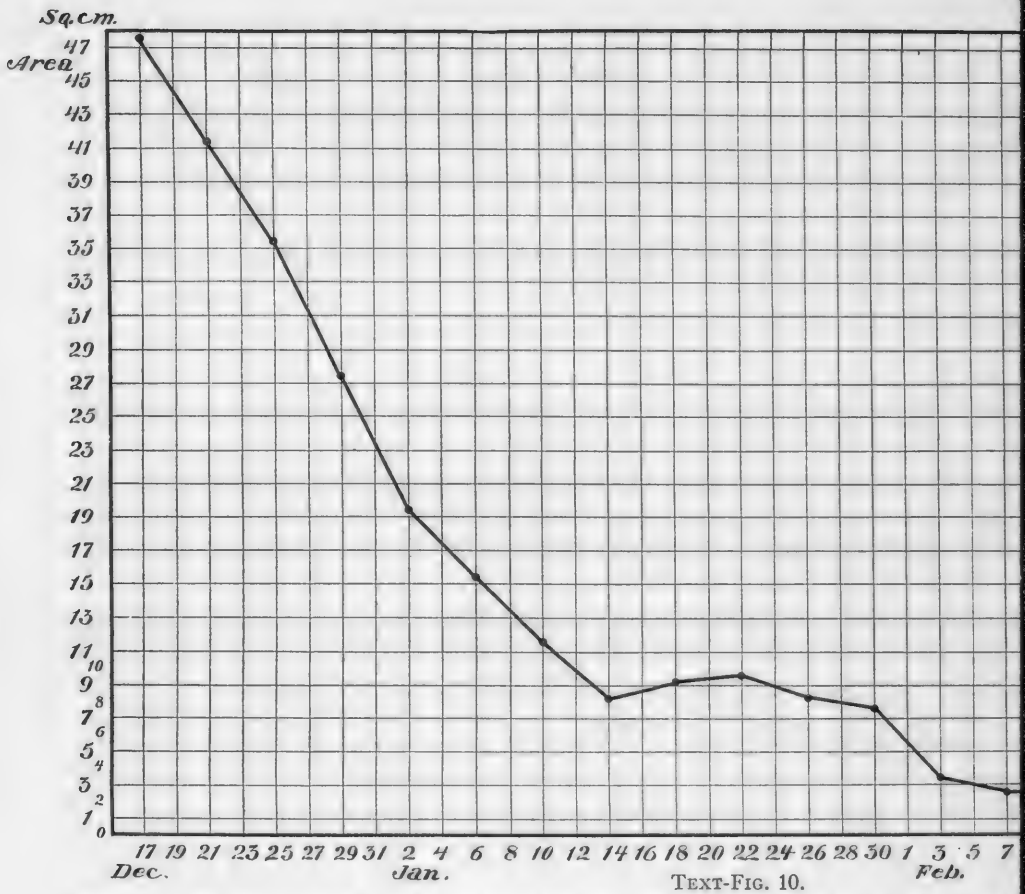
The curves representing the progress of cicatrization in these experiments assume a geometric appearance. It seemed probable, therefore, that the relation between the size of a wound and the rate of repair may be expressed mathematically.[3]

The regularity of cicatrization depends in a large measure on the bacteriologic condition of the wound. The more aseptic the wound, the more regular is the curve of cicatrization. In Experiment 1 the wound was aseptic during the greater part of the period of repair. Agar and bouillon, inoculated with the secretions of this wound, remained sterile. In the other experiments the wounds were slightly infected. After a wound was chemically sterilized the rate of cicatrization increased (Text-fig. 9).

When an aseptic or slightly infected wound was infected, the curve of cicatrization became horizontal or inflected upwards, showing that arrest or retrogression of the repair occurred. In the following experiment a wound accompanying a fracture of the humerus had been almost completely sterilized and was cicatrizing normally, when a slight infection occurred. Cicatrization stopped and the wound enlarged (Text-fig. 10).

Some of the sterile wounds became infected while under observation. As soon as bacteria appeared the process of repair was retarded

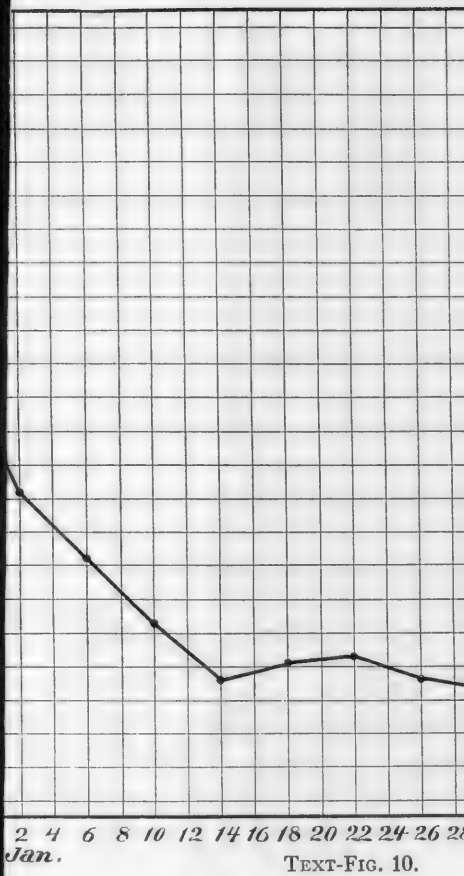
[3] du Noüy, P., *J. Exp. Med.*, 1916, xxiv, 451.



TEXT-FIG. 10.

Experiment 10.—Patient 266, age 33 years (Text-fig. 10). Wound and fracture of the left arm. The
On Jan. 14 the infection caused the curve to become irregular.

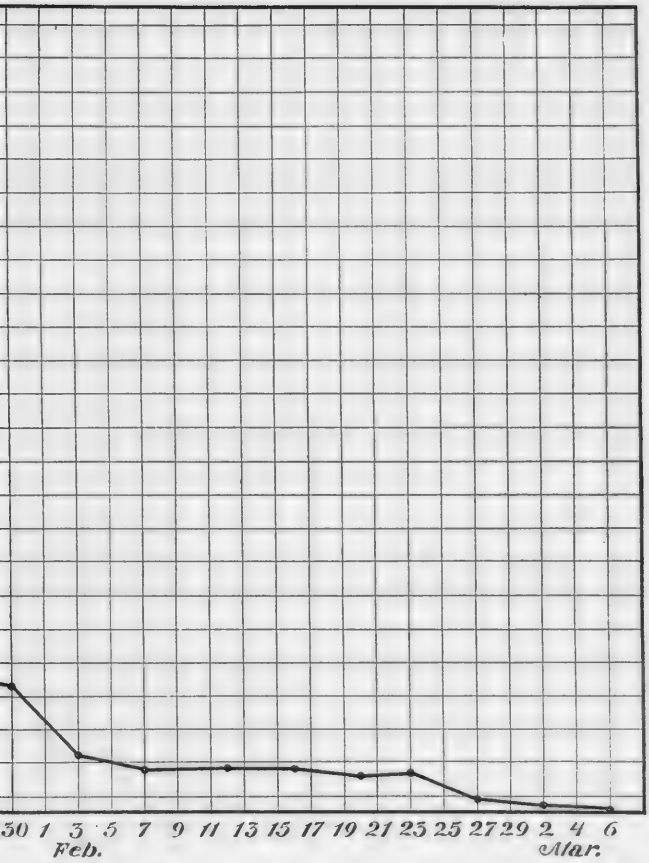
	Dec. 17	21	25	29	Jan. 2	6	10	14	18
S.....	47.3	41.3	35.4	27.3	19.4	15.4	11.7	8.2	9.2
R.....		1.5	1.5	2.0	1.97	1.0	0.92	0.87	0.2



TEXT-FIG. 10.

e 33 years (Text-fig. 10). Wound and fracture of
e curve to become irregular.

	Dec. 17	21	25	29	Jan. 2	6	
S.....	47.3	41.3	35.4	27.3	19.4	15.4	1
R.....		1.5	1.5	2.0	1.97	1.0	0



he left arm. The wound was slightly infected from Dec. 17 to Jan. 14.

0	14	18	22	26	30
.7	8.2	9.2	9.6	8.3	7.6
92	0.87	0.2	0.1	0.3	0.1

and sometimes stopped. However, infection, when it was of short duration, had no marked disturbing influence on the curve representing the cicatrization of the wound. As soon as normal conditions were reestablished, the progress of cicatrization was hastened (Text-fig. 9). After a period of inactivity an automatic mechanism seemed to accelerate the healing process. This explains why short periods of infection did not produce marked irregularities in the curve of cicatrization. Again, a period of greater activity was generally followed by a period of slow repair which reestablished the regularity of the curve.

In all the experiments cicatrization was more rapid at the beginning than at the end of the period of repair. At the beginning of the process of contraction, the curve inclined rapidly downwards (Text-fig. 7), then the inclination diminished and finally the curve became almost horizontal. The greater activity of the beginning of the granulating period has already been observed. It was generally attributed to the aging of the wound. In previous experiments[1] we found that during the period of contraction, a wound 60 to 70 mm. wide diminished from 9 to 10 mm. in 24 hours. When it was not more than 20 mm. wide the velocity of cicatrization decreased markedly and became very slight when the edges of the wound were located at a distance of only 10 to 12 mm. from each other. In recent experiments the variations of the size of a wound and not only of one of its dimensions were examined. The absolute value of the rate of cicatrization, that is, the surface lost by the wound in 24 hours, depended mainly on the size of the wound. Wounds of from 120 to 150 sq. cm. decreased from 4 to 7 sq. cm. in 24 hours, while the daily diminution of wounds from 10 to 20 sq. cm. was but 1 to 2 sq. cm. In Experiment 1 the wound followed the same law. When its area was 16.2 sq. cm., it diminished at a speed of 1.37 sq. cm. When it attained 1 sq. cm. its velocity became 0.3 sq. cm. It was therefore evident that the rate of repair was proportional to the size of the wound.

The rate of cicatrization, however, was not exactly proportional to the size. For example, in Experiment 1 the area which on Dec. 17 was 16.2 sq. cm. became 1 sq. cm. on Jan. 6. If the equation

$$\frac{S}{S'} = \frac{R}{R'}$$

represented the rate of cicatrization, the rate R' would be expressed thus:

$$R' = \frac{RS'}{S}$$

and its value in Experiment 1 would be $R' = 0.08$. However, it was found that the rate of cicatrization when the size of the wound was 1 sq. cm. decreased only to 0.3 instead of 0.08, and that the complete healing occurred at the end of 6 days. Similar results having been obtained in other experiments it may be concluded that the rate of cicatrization diminishes at the same time as the size but less rapidly. On the other hand, comparison of the figures representing the rate and the area shows that the value of the rate in relation to the size augmented progressively. In Experiment 1 the velocity of cicatrization was successively $\frac{1}{36}$, $\frac{1}{11}$, $\frac{1}{10}$, $\frac{1}{11}$, $\frac{1}{8}$, $\frac{1}{7}$, $\frac{1}{3}$, $\frac{1}{5}$, and $\frac{1}{2}$ of the area. It was found also that the value of the rate in relation to the surface was always greater in a small than in a large wound. If it were otherwise cicatrization would be extremely slow and wounds would not heal.

As stated above, the diminution of the velocity of cicatrization as healing progresses has been generally attributed to the aging of the wound. In reality the phenomenon depends on the reduction of the size of the wound. Former experiments have shown that during a similar period large wounds cicatrized with greater velocity than small ones. In recent experiments made on men and on guinea pigs the rates of cicatrization of wounds of equal area and of different age were compared. Table I shows that wounds of an area of 2 sq. cm. cicatrized completely in from 8 to 10 days and that no relation existed between their age and the velocity of cicatrization.

TABLE I.

Case No.	Age of wound.	Size of wound.	Day of healing.
		sq. cm.	
290	42 days.	2.2	8
354	7 mos.	2.2	8
256	2 "	2	9
330	13 days.	2.1	8
217	2 mos.	2.2	10
221	5 "	2.2	8

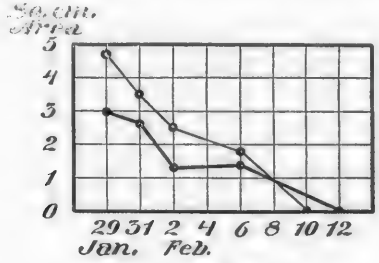
The observations made on guinea pigs gave similar results (Table II).

TABLE II.

Guinea pig No.	Age of wound.	Size of wound.	Day of healing.
	days	*sq. cm.*	
1	7	1.1	8
5	5	1.1	6
2	11	1.1	4
3	11	1.1	5

Since wounds of equal size but of different age cicatrized with variable velocity independently of the age of the wound, the slackening in the speed observed at the end of the period of cicatrization was probably the result of the diminution of the size.

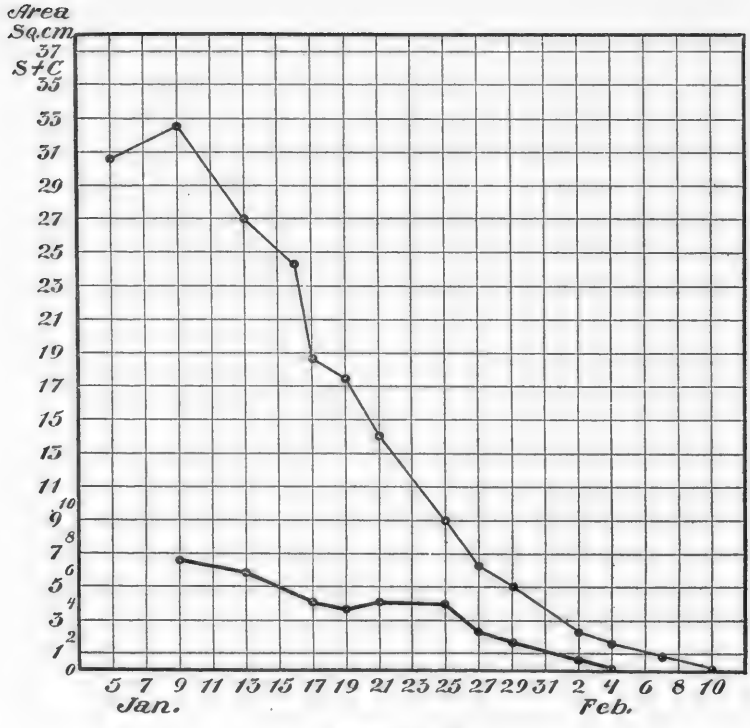
To complete the demonstration of this fact, we have studied wounds of equal age and unequal size. Previous experiments had shown that if two wounds were made at the same time on a dog, the rate of repair of the larger wound was greater than that of the smaller one. So in trapezoidal wounds the reduction of the smaller side was slower than the diminution of the larger side. After a few days, therefore, a trapezoidal wound showed a tendency to become rectangular, circular, or oval. These results were confirmed in the following experiments.



TEXT-FIG. 11.

Experiment 11.—Patient 286, age 30 years (Text-fig. 11). Shell wounds of the clavicular region. The interval between the curves of the larger and smaller wounds became progressively shorter, showing that the rate of repair of the larger wound was greater than that of the smaller one. The larger wound healed before the smaller one.

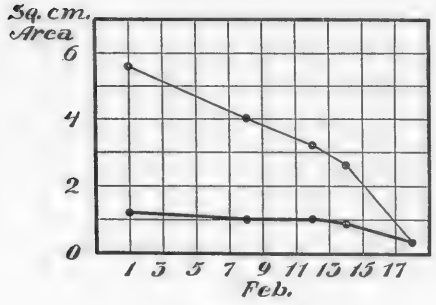
	Area (S) of larger wound.	Area (S') of smaller wound.	$S-S'$	$\frac{S}{S'}$
Jan. 29	4.7	3.0	1.7	1.5
Feb. 6	1.7	1.4	0.3	1.2



TEXT-FIG. 12.

Experiment 12.—Patient 290, age 31 years (Text-fig. 12). Shell wounds with fracture of the radius and cubitus. The curves of both wounds tend to unite.

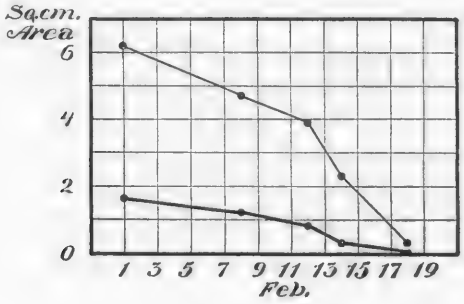
	Area (S) of larger wound.	Area (S') of smaller wound.	$S-S'$	$\frac{S}{S'}$
Jan. 9	32.5	6.7	25.8	4.8
Feb. 2	2.3	0.6	1.7	3.8



TEXT-FIG. 13.

Experiment 13.—Cat 1 (Text-fig. 13). Wounds of the abdominal wall. The curves of both wounds unite at the end of the period of cicatrization.

	Area (S) of larger wound.	Area (S') of smaller wound.	$S-S'$	$\frac{S}{S'}$
Feb. 1	5.6	1.2	4.4	4.6
" 18	0.3	0.3	0	1



TEXT-FIG. 14.

Experiment 14.—Cat 2 (Text-fig. 14). Wounds of the abdominal wall. The curves of both wounds tend to unite.

	Area (S) of larger wound.	Area (S') of smaller wound.	$S-S'$	$\frac{S}{S'}$
Feb. 1	6.2	1.6	4.6	3.8
" 18	0.3	0	0.3	

The graphic expression of these experiments shows that wounds of unequal dimensions have a tendency in the course of cicatrization to become equal in curve. In Experiment 13 a wound of 5.6 sq. cm. and another wound of 1.2 sq. cm. were both reduced after 17 days to

0.3 sq. cm. (Text-fig. 13). It is evident that the lessening in the rate of cicatrization at the end of the period of repair is due to the progressive diminution of the size of the wound and not to its age.

The Value of Contraction and of Epidermization in the Healing of Wounds.

In order to study the relation of contraction and epidermization and the comparative value of both processes, not only the area of the wound had to be known but also that of the cicatrix. The area of the cicatrix was determined by the use of a planimeter on cellophane tracings. The curves expressing the area of the wound and the area of the cicatrix and the wound were separated by an interval which represented in square centimeters the area of the cicatrix at a given time. The value of the epidermization and of the contraction could thus be ascertained every day.

In these experiments errors might arise from lack of distinctness in the outer edge of the cicatrix. It was necessary, therefore, to select wounds whose measurements could be made with sufficient accuracy. The experiments were made on eight wounds on patients of different ages, on a wound made on a guinea pig, and on two wounds made on cats.

Experiment 23.—Cat 1. Wound of the abdominal wall.

	Feb. 1	8	12	14	18	23
S	5.6	4.0	3.2	2.6	0.35	0
S + *C*	5.6					1.3

Experiment 24.—Cat 2. Wound of the abdominal wall.

	Feb. 11	12	14	18	21	23
S	6.2	3.9	2.3	0.35	0.1	0
S + *C*	6.2					0.5

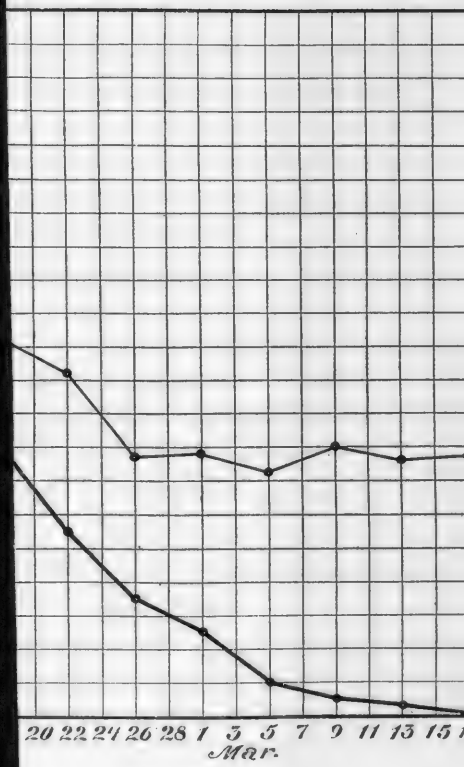
Experiment 25.—Patient 354, age 40 years. Old wound on a stump.

	Jan. 20	22	24	25	27	30	Feb. 1	4	6
S	6.2	4.7	3.5	3.0	2.2	1.3	1.0	0	
S + *C*	42.8				40.0		37.0		40.7

TEXT-FIG. 15.

Experiment 15.—Patient 361, age 21 years (Text-fig. 15). Shell wound of the posterior thoraci
The lower curve represents the area of the wound, and the upper curve the area of both wound and
The curve of the wound was horizontal until Feb. 6. Afterwards it descended regularly. The upp
became horizontal on Feb. 26, showing that the last part of the healing was due to epidermizat
and not to contraction.

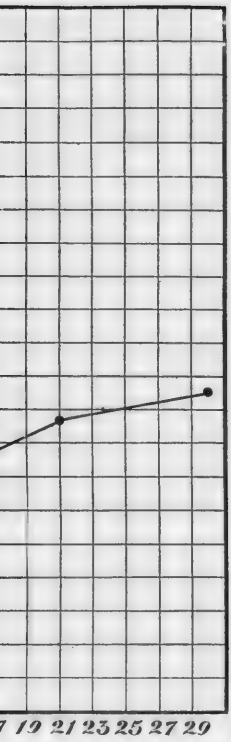
	Feb. 2.	6	10	14	16	18	22	26	Mar. 1
S.....	40.2	40.0	29.3	24.0	19.8	16.0	11.0	7.1	5.0
S + *C*.....						22.5	20.5	15.5	15.6



TEXT-FIG. 15.

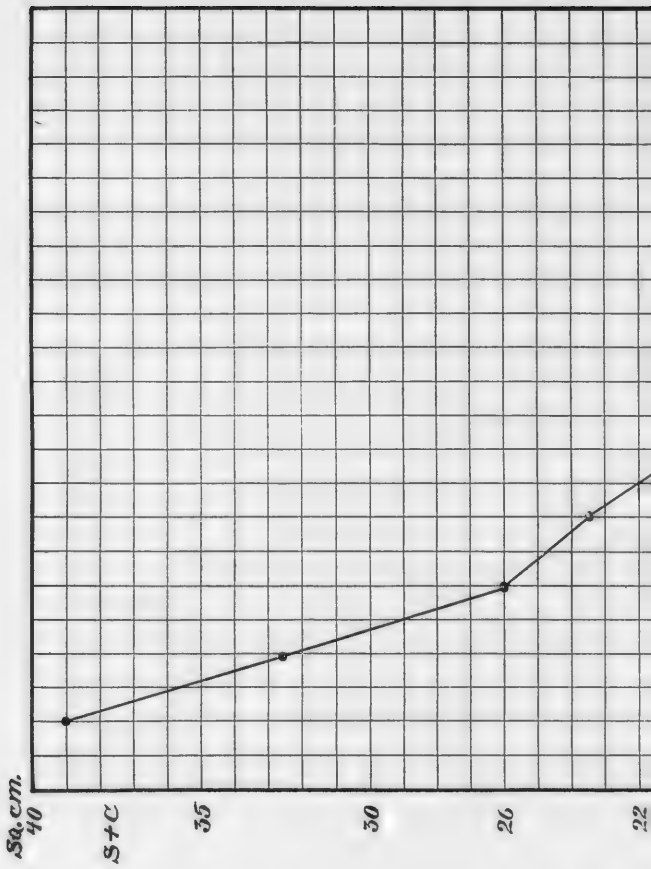
21 years (Text-fig. 15). Shell wound of the
of the wound, and the upper curve the area of
ntal until Feb. 6. Afterwards it descended reg
wing that the last part of the healing was du

2.	6	10	14	16	18	22
2	40.0	29.3	24.0	19.8	16.0	11.0
					22.5	20.5



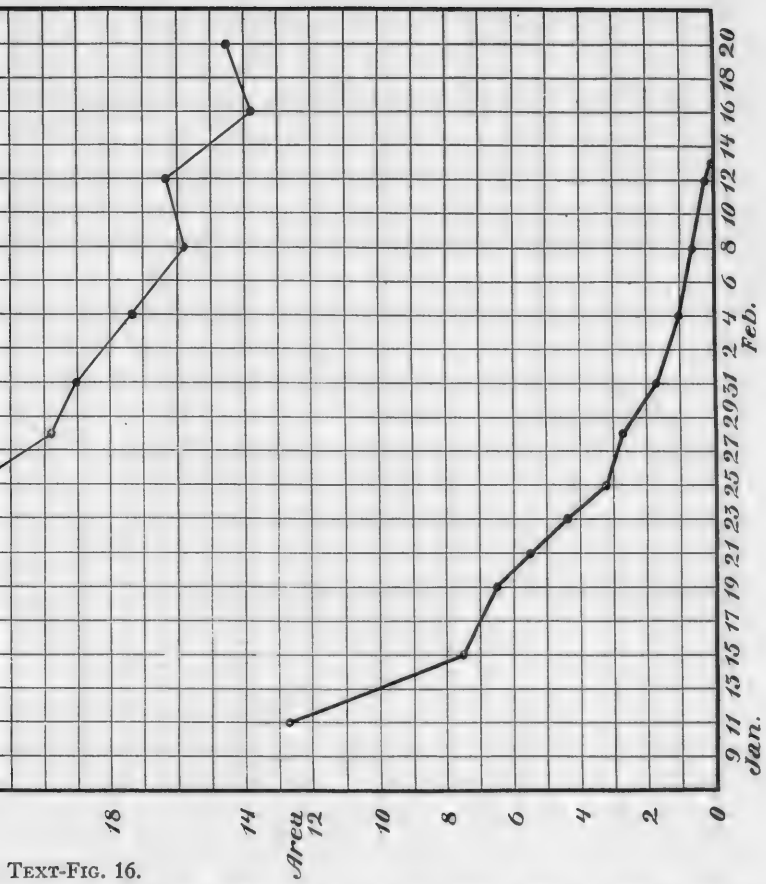
osterior thoracic region.
oth wound and cicatrix.
larly. The upper curve
to epidermization alone

26	Mar. 1
7.1	5.0
5.5	15.6



Experiment 16.—Patient 256, age 21 years (Text-fig. 16). Sh
shows that the wound cicatrized at the same time by both proces
curve ascended, because the cicatrix began to repair.

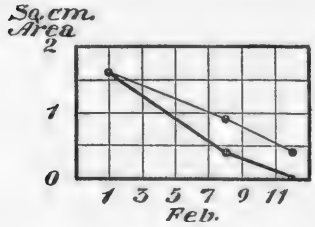
	Jan. 11	15	19	21	
S.....	12.7	7.5	6.5	5.5	
$S + C$	39.0	32.5	26.0		



TEXT-FIG. 16.

ll wound of the leg. Both upper and lower curves descend together. This es, contraction and epidermization. After healing was completed, the upper

23	25	28	31	Feb. 4	8	12	13
4.4	3.2	2.7	1.7	1.0	0.6	0.2	0
3.5		19.8	19.0	17.4	15.8	16.3	



TEXT-FIG. 17.

Experiment 17.—Guinea Pig 5 (Text-fig. 17). Wound of the abdominal wall. Both curves descend together showing that epidermization and contraction took part in the healing of the wound.

	Feb. 1	8	12
S	1.6	0.4	0
S + *C*	1.6	0.9	0.4

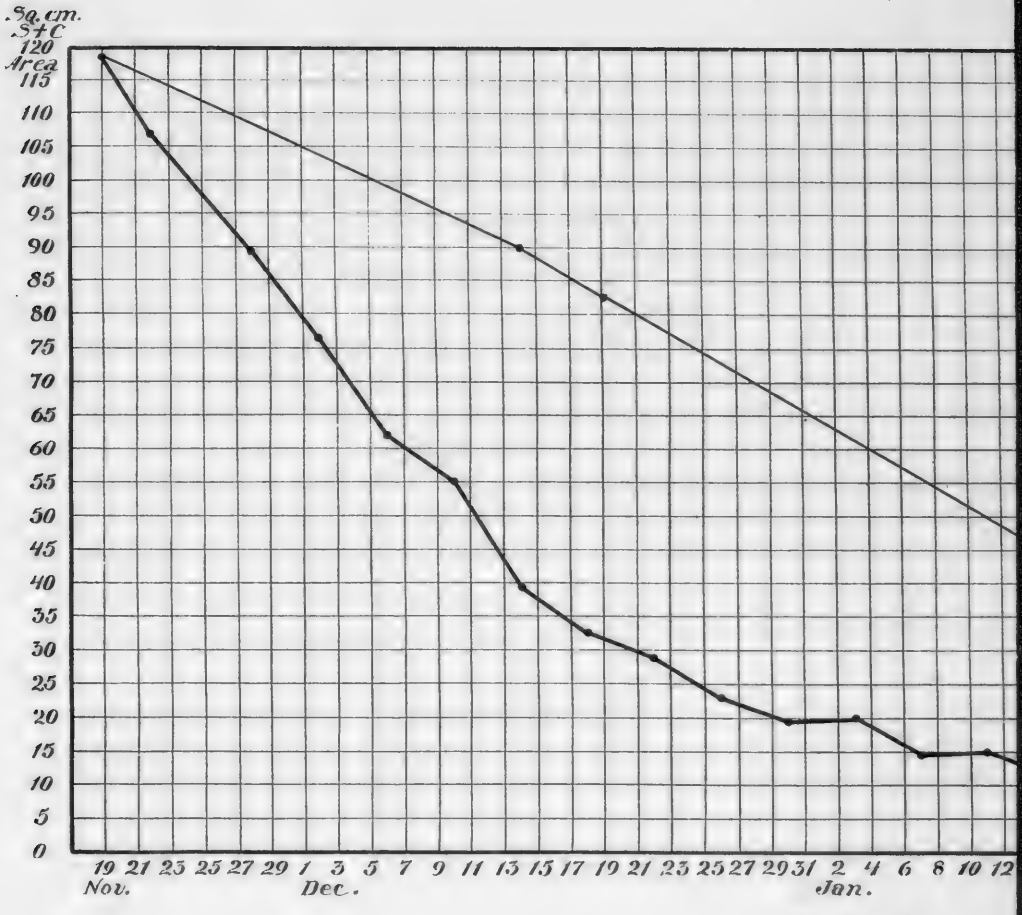
The tracings representing the cicatrization of these wounds demonstrate that contraction was nearly always associated with epidermization. In the experiments previously made on dogs at The Rockefeller Institute, the period of contraction started from 2 to 5 days after the resection of the flap of skin. The beginning of the contraction was preceded by a period during which the tracing had a horizontal direction. Then the curve inflected abruptly downwards and the reparation of the wound began. In Experiment 7 the period of immobility of the wound lasted for 6 days. In Experiment 15 it lasted longer although the wound was covered with granulations. A fragment of shell, remaining in the deep part of the wound, maintained the tissues in a condition of infection which prevented reparation.

After a few days the processes of contraction and epidermization always coexisted. However, there was a period when the wound healed exclusively by contraction. On the curve of Experiment 15 it is seen that during 12 days, contraction alone caused the reparation. Afterwards, epidermization started and the wound cicatrized under the influence of both processes. In most of the wounds observed on human beings contraction and epidermization worked together. Generally contraction continued until complete healing. The curves of the wounds in Experiments 16, 18, and 25 illustrate this phenomenon. However, contraction sometimes stopped before cicatrization was complete. It should be remembered that this process is dependent on contraction of the granulation tissue and on the opposite action of

the tissues surrounding the wound. The resistance of the skin to granulous contraction is very slight in narrow wounds. If the defect is larger, the wound contracts until the elasticity of the surrounding skin prevents a greater reduction of its size. It is also probable that under certain conditions the granulation tissue does not possess the power of contraction. In Experiment 18 contraction did not last until complete healing. The curves representing the cicatrization of the wounds of Experiments 21 and 22 show that during the last days of repair the area of the cicatrix enlarged. In both wounds epidermization assumed almost entirely the effort of repair. As contraction was lacking, epidermization became more rapid and compensated for the dilatation of the cicatrix. It would seem that the processes of contraction and epidermization, although independent in some measure, can each make up for the other's deficiency.

Contraction always activated healing unless retrogression of epidermization occurred simultaneously. The upper curve of Experiment 23 shows a sudden and temporary acceleration of contraction. The lower curve remains parallel to the upper curve. It shows that epidermization had no influence on the rate of contraction. The lower curve indicates a sudden acceleration of the cicatrization, under the influence of a certain substance. During the same period the upper curve remained regular, showing that contraction pursued its normal course while the process of epidermization alone responded to the disturbing action of the dressing. The factors which acted on one of the processes appeared to have been without influence on the other.

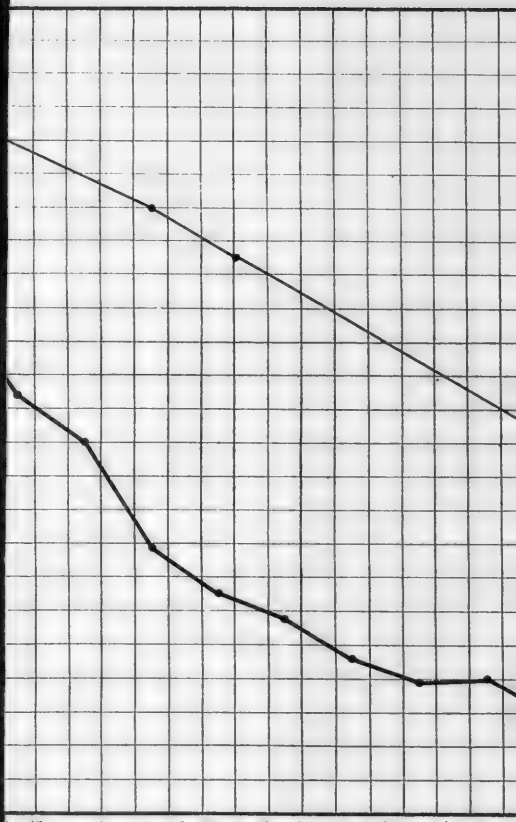
The relative importance of both processes, contraction and epidermization, was valued quantitatively by comparing the area of the cicatrix and that of the wound. The area of the cicatrix represents the result of the epidermization. The difference between the surface of the cicatrix and of the wound gives the value of the contraction. Previous experiments had shown that the cicatrix of a large wound was comparatively smaller than that of a small wound. Two rectangular wounds, one 66 mm. wide and the other 26 mm. wide, were made on the same dog under ether anesthesia. The wound of 66 mm. gave a cicatrix 22 mm. wide, and that of 26 mm. a cicatrix of 13 mm. The width of the cicatrix of the larger wound represented a third of



TEXT-FIG. 18.

Experiment 18.—Patient 263, age 36 years (Text-fig. 18). Wound of the forearm with fracture of soon as the healing was completed, the cicatrix became larger, as is shown by the upward inflection

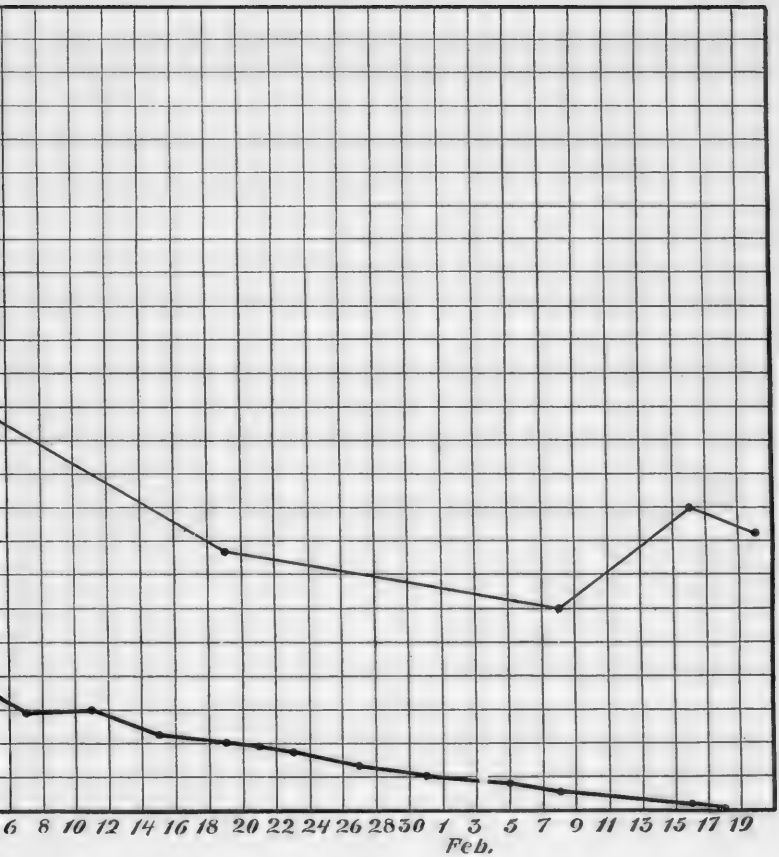
	Nov. 19	28	Dec. 6	14	26	Jan. 7	19
S.....	118.5	89.6	62.1	39.7	23.0	14.8	10.0
S + *C*.....				90.0			38.5



TEXT-FIG.

age 36 years (Text-fig. 18). Wound of the forearm wi
ed, the cicatrix became larger, as is shown by the upw

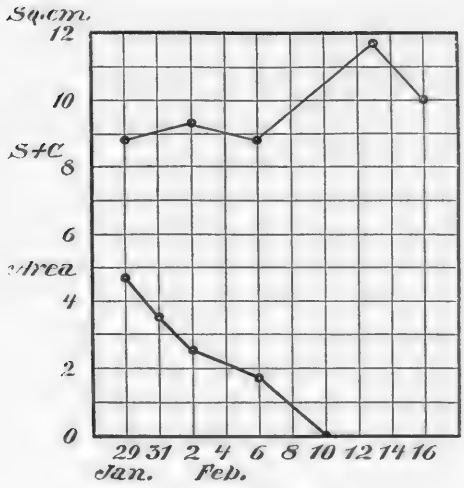
	Nov. 19	28	Dec. 6	14	26
S.....	118.5	89.6	62.1	39.7	23.0
$S + C$.....				90.0	



18.

h fracture of the cubitus. Cicatrization is brought about by both processes. As rd inflection of the curve.

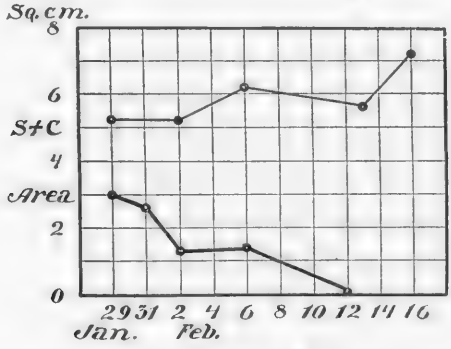
Jan. 7	19	31	Feb. 8	16	20
14.8	10.0	5.2	2.6	0.4	0
	38.5		30.0		41.0



TEXT-FIG. 19.

Experiment 19.—Patient 286, age 30 years (Text-fig. 19). Wound of the upper clavicular region. The upper curve remains horizontal for a while and afterwards goes up. Cicatrization was due entirely to epidermization and not to contraction.

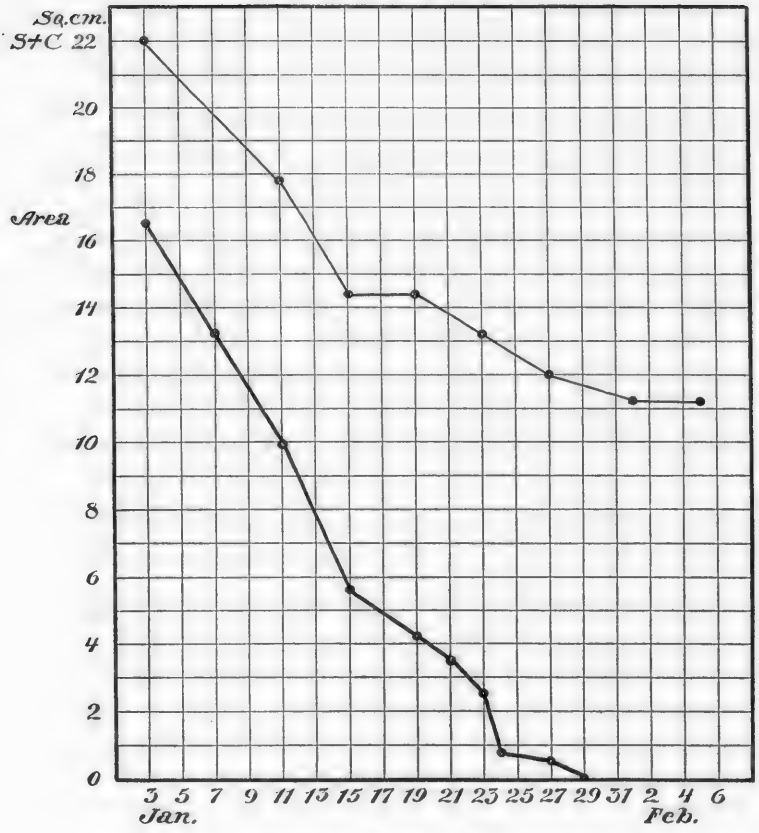
	Jan. 29	31	Feb. 2	6	13
S.....	4.7	3.5	2.5	1.7	0
S + *C*.....	8.8		9.3	8.8	11.7



TEXT-FIG. 20.

Experiment 20.—Patient 286, age 30 years (Text-fig. 20). Wound of the lower clavicular region. The upper curve remains almost horizontal. No contraction. Cicatrization was produced by epidermization.

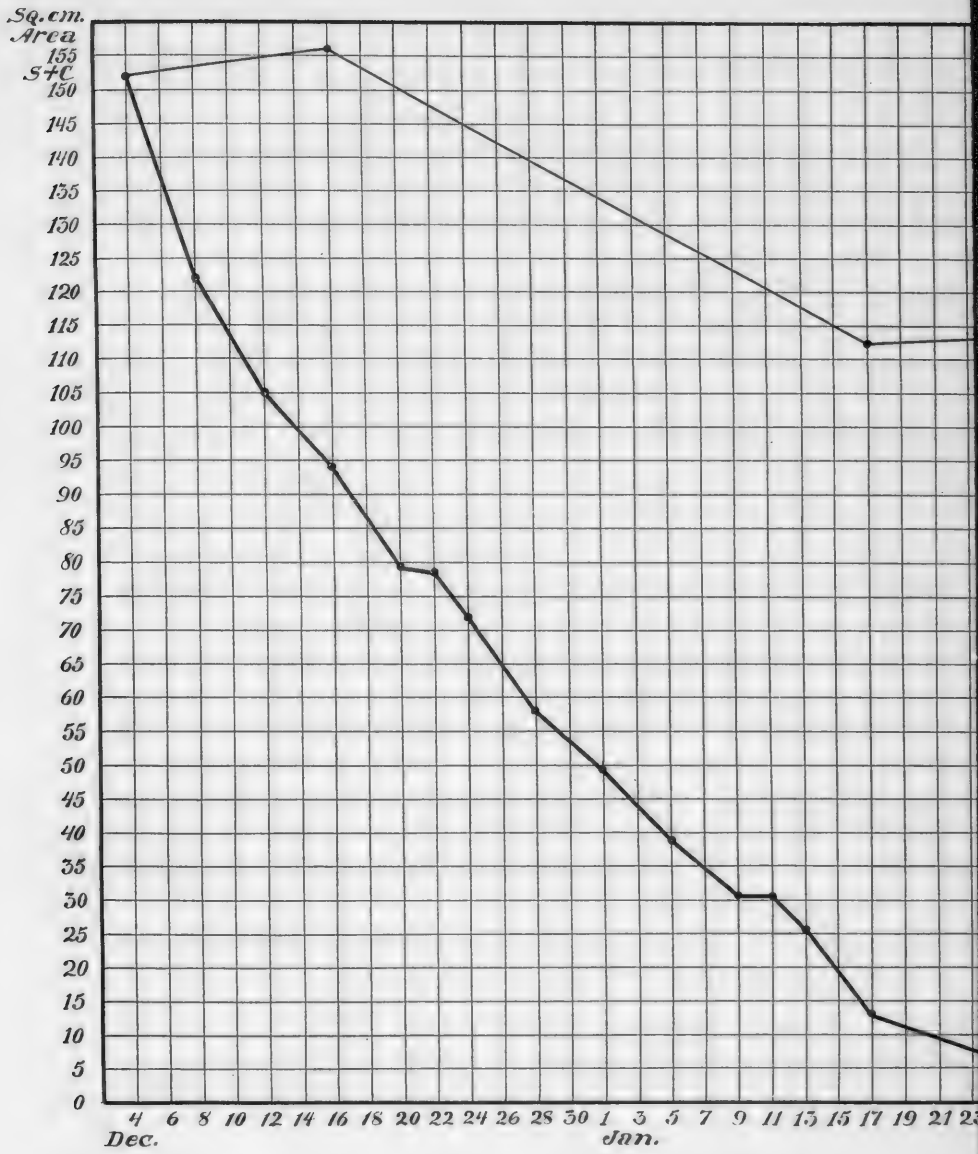
	Jan. 29	31	Feb. 2	6	12	13	16
S.....	3.0	2.6	1.3	1.4	0		
S + *C*.....	5.2		5.2	6.2		5.6	7.2



TEXT-FIG. 21.

Experiment 21.—Patient 289, age 29 years (Text-fig. 21). Shell wound of the external part of the leg. On Jan. 15 the downward inflection of the lower curve was the result of the acceleration of contraction. From Jan. 15 to 19, the upper curve remained horizontal while the lower curve went downward. On Jan. 24 the lower curve was irregular while the upper curve was not disturbed, showing that both contraction and epidermization were in some measure independent.

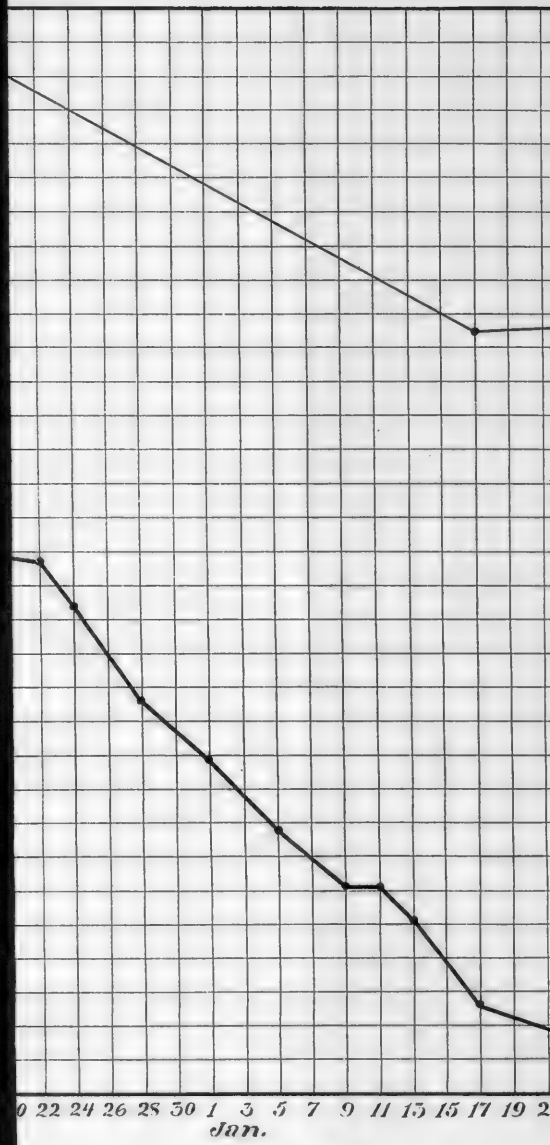
	Jan. 3	7	11	15	19	21	23	24	27	29
S.....	16.5	13.2	9.9	5.6	4.2	3.5	2.5	0.8	0.5	0
$S+C$....	22.0		17.8	14.4	14.4		13.2		12.0	



TEXT-FIG. 22.

Experiment 22.—Patient 269, age 35 years (Text-fig. 22). Burn of the external surface c
curves shows that cicatrization was produced chiefly by epidermization. After Jan. 17 contr

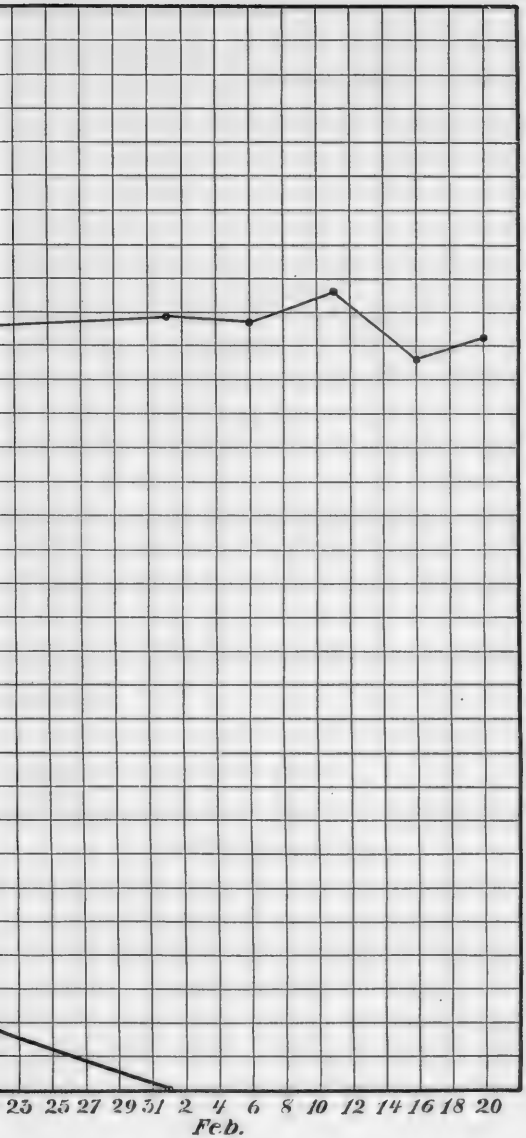
	Dec. 4	8	16	24	Jan. 5
S.....	152.0	122.2	94.0	72.0	39.0
S + *C*.....	152.0		156.0		



TEXT-FIG. 22.

age 35 years (Text-fig. 22). Burn of the external surfa
as produced chiefly by epidermization. After Jan. 17 c

	Dec. 4	8	16	24	Ja
S	152.0	122.2	94.0	72.0	39
S + C	152.0		156.0		



e of the right arm. The large interval between both
ntraction ceased altogether.

5	17	Feb. 5	11
0	12.8	0	0
	112.5	113.5	118.0

the width of the wound, and that of the smaller only a half. The cicatrix of a wound of about 1 sq. cm. in size was scarcely smaller than the wound. These facts were confirmed by Experiments 17, 24, 23, and 20. On Guinea Pig 6 three-quarters of the cicatrization was due to the contraction, and the last quarter to epidermization. In Cat 3 both processes acted in similar proportion. In Cat 2 one-twelfth of the cicatrization was due to epidermization. On the contrary, the reparation of the wounds in Experiments 19 and 20 was caused entirely by epidermization. As a rule, contraction played a more important part in healing than epidermization.

Mensuration of the cicatrix at the end of the period of repair showed that contraction stopped as soon as the wound was completely healed. From this time, the size of the cicatrix increased progressively. Thus, the period of contraction was followed by a phase of expansion, as in Text-fig. 15. The wound of Guinea Pig 6, the width of which was 4 mm. at the time of healing, measured 7 mm. 5 days later. The area of the cicatrix of Patient 354 in Experiment 25, which was 37sq. cm. the day of healing, reached 40 sq. cm. 5 days later. The cicatrix of Patient 269 which was 113.5 sq. cm. had an area of 118 sq. cm. 6 days later. Expansion was much less marked in man than in the dog. In animals whose experimental wounds were produced by resection of a large flap of skin, the dilatation of the cicatrix was much larger. It spread slowly for a long time in such a way that the scar might become as large as the original wound.

CONCLUSIONS.

1. A method for measuring the area of a wound not geometric in form is described.

2. The rate of cicatrization of a wound is greater at the beginning than at the end of the period of repair. It depends on the area rather than on the age of the wound. There is a constant relation between the size of a wound and the rate of cicatrization. The larger the wound the greater is the rate of cicatrization. Two wounds of different size have a tendency to become equal.

3. The rate is proportional to the area, but diminishes less rapidly than the area.

4. The process of contraction is the more important factor in the repair of a wound. Epidermization completes the work of contraction. After the wound is healed, the cicatrix as a rule expands.

5. The curve representing the diminution of the size of an aseptic wound while it cicatrizes is regular and geometric.

CICATRIZATION OF WOUNDS.

II. Mathematical Expression of the Curve Representing Cicatrization.

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(From the Laboratories of The Rockefeller Institute for Medical Research, New York, and Hospital 21, Compiègne, France.)

(Received for publication, May 24, 1916.)

In order to study the process of cicatrization, a technique for measuring accurately the area of wounds was developed. Sterilized cellophane was applied to the wound and the edge was outlined with a wax pencil. This drawing was transferred in ink to an ordinary sheet of paper. Afterwards the area was measured by means of a planimeter, either the Amsler system or some other. A curve was obtained by carrying the area, in square centimeters, in ordinates, and the time, in days, in abscissæ.

In many experiments made by Dr. Carrel the curve representing the cicatrization of aseptic wounds was of regular and geometric appearance. These curves were expressed by a mathematical equation in function of time and area.

After a large number of slightly infected wounds had been studied, a simple extrapolation formula was obtained. Marked deviation from the calculated curve showed generally that infection had set in. By means of the formula the area of the wound after a given time can be foreseen. The formula may be expressed in two equations.

$$(1) \quad \frac{\frac{S - S'}{S}}{t + \sqrt{T}} = i$$

$$(2) \quad S'' = S' [1 - i (t' + \sqrt{T + t'})]$$

S represents the area of the wound at the beginning of the experiment.

S' represents the area of the wound t days later, at the time of the second observation. (We may say 4 days, in order that the area cicatrized, $S-S'$, may be of sufficient size.)

t represents the time elapsed between the first two observations, S and S', in days.

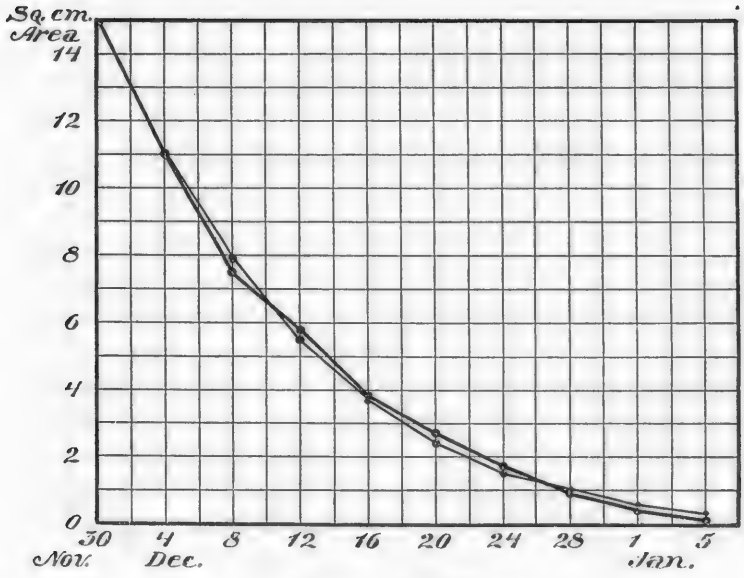
T represents the age of the wound from the time of the first observation S. Therefore in the formula (1) $T = t$, and practically $t = t' = 4$ days.

t' represents the time between the last observation S' and the time of the theoretical surface S'' of the wound.

i is a constant coefficient characterizing each wound.

Thus the first equation tends to establish an index of cicatrization i which is carried into the second in order to calculate the surface of the wound at a given time. It should be noticed that the best approximation is obtained when the time t is the same as the time t'. Therefore, after two observations, 3 or 4 days apart, of a wound which heals aseptically, the area that it should have 4, 10, 20, or 30 days later, and the day on which it should be healed can be computed. Accidents, of course, may retard temporarily the progress of the phenomenon. But generally the time lost is regained by a rapid acceleration as shown in Wounds 4, 6, and 7.

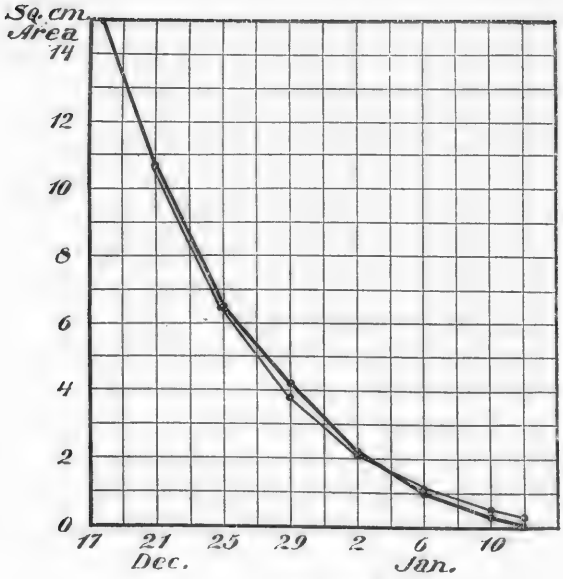
In the following experiments the calculated areas are compared with the observed areas.



TEXT-FIG. 1.

Experiment 1.—Patient 217, age 37 years. Wound of the arm. On Nov. 30, the size was 15.3 sq. cm. Index = 0.041 (Text-fig. 1). Between Dec. 18 and 24, a few bacteria appeared on the films (1 to 3 per microscopic field).

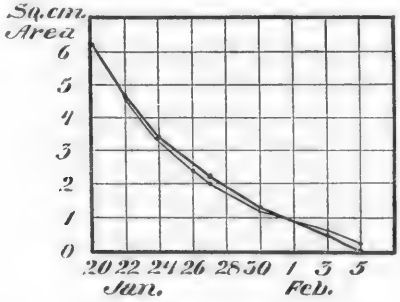
	1915 Dec. 4	8	12	16	20	24	28	1916 Jan. 1	5
Observed area..	11.0	7.5	5.8	3.8	2.7	1.7	0.9	0.4	Cicatrization.
Calculated "..		7.9	5.5	3.7	2.4	1.55	0.96	0.58	"



TEXT-FIG. 2.

Experiment 2.—Patient 221, age 27 years. On Dec. 17 the area of the wound was 16.2 sq. cm. Index = 0.057 (Text-fig. 2). There was a slight infection between Dec. 27 and 29 (1 microorganism in two microscopic fields).

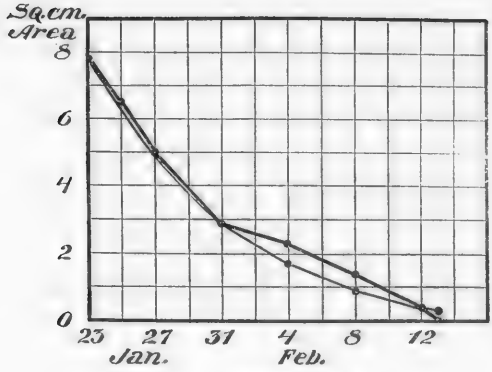
	1915 Dec. 21	25	29	1916 Jan. 2	6	10	12
Observed area.....	10.7	6.5	4.2	2.2	1.0	0.3	Cicatrization.
Calculated "		6.5	3.8	2.1	1.1	0.5	"



TEXT-FIG. 3.

Experiment 3.—Patient 354, age 40 years. On Dec. 20 the area of the wound was 6.2 sq. cm. Index = 0.07 (Text-fig. 3).

	1916 Jan. 22	24	27	30	Feb. 3	5
Observed area.....	4.7	3.4	2.2	1.3	0.5	Cicatrization.
Calculated "		3.4	2.4	1.2	0.54	"

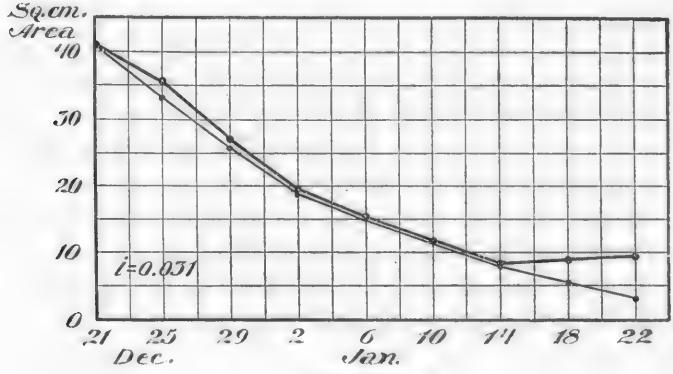


TEXT-FIG. 4.

Experiment 4.—Patient 330, age 31 years. On Jan. 23 the area of the wound was 7.8 sq. cm. Index = 0.059 (Text-fig. 4).

	1916 Jan. 25	27	31	Feb. 4	8	12	13
Observed area.....	6.5	5.0	2.9	2.3	1.4	0.4	Cicatrization.
Calculated "		5.0	2.98	1.7	0.9	0.44	"

Between February 3 and 8 the wound became infected and deviated from the theoretical curve. However, it overtook the latter on February 12 after it was again sterilized. This shows that acceleration of the cicatrization has occurred, which always happened in the cases which we studied.

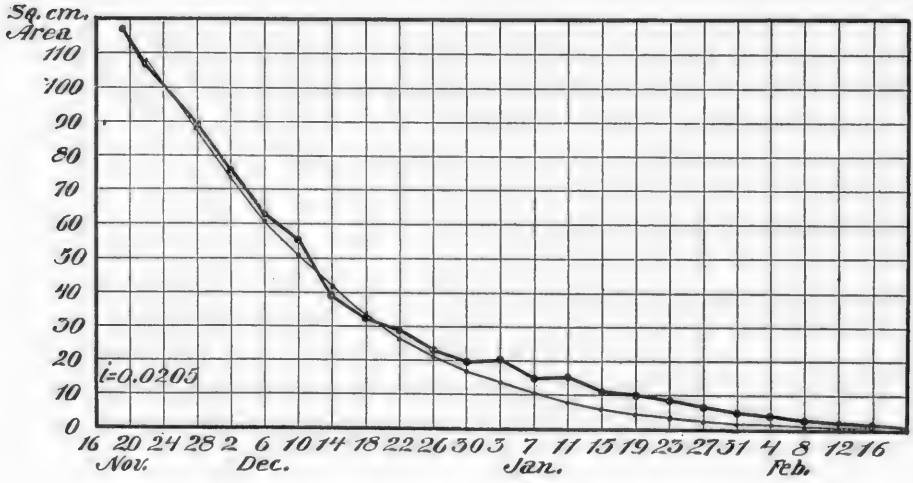


TEXT-FIG. 5.

Experiment 5.—Patient 266, age 33 years. On Dec. 25 the area of the wound was 35.4 sq. cm. Index = 0.031 (Text-fig. 5). On Jan. 14 the wound became infected and began to increase in size. It deviated from the theoretical curve and did not meet it again.

	1915 Dec. 29	1916 Jan. 2	6	10	14	18
Observed area.....	27.3	19.4	15.4	11.7	8.2	9.0
Calculated "		19.1	15.6	11.3	8.0	5.6

The following experiment was similar, but the observed curve coincided with the calculated curve before healing. The date of healing could be predicted 90 days in advance.

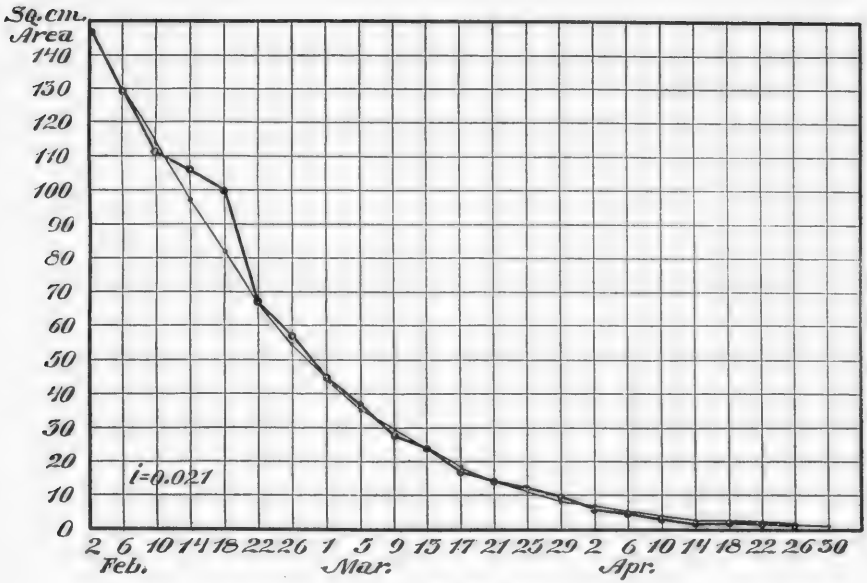


TEXT-FIG. 6.

Experiment 6.—Patient 263, age 36 years. On Nov. 19 the area of the wound was 118.5 sq. cm. Index = 0.0205 (Text-fig. 6). The fluctuations of the curve were due to infection. The observed curve has, as always, a tendency to regain time lost in order to rejoin the calculated curve (from Dec. 10 to 14, then from Jan. 27 to Feb. 18).

	1915 Nov. 22	28	Dec. 2	6	10	14	18	22	26	30
Observed area.....	107.0	89.6	76.0	62.1	55.1	39.7	32.5	29.1	23.0	19.5
Calculated "		88.0	74.2	61.8	51.0	41.6	33.6	26.9	21.3	16.8

	1916 Jan. 3	7	11	15	19	23	27	31	Feb. 4	8	12	16	18
Observed area.....	20.0	14.8	15.0	11.0	10.0	8.5	6.5	5.2	4.3	2.6	1.7	0.4	Cicatrization.
Calculated area..	13.1	10.1	7.8	5.9	4.5	3.4	2.5	1.9	1.4	1.0	0.74	0.5	"



TEXT-FIG. 7.

Experiment 7.—Patient 360, age 22 years. Index = 0.021 (Text-fig. 7). The deviation at the beginning (Feb. 18) was due to infection. After sterilization it overtook the calculated curve.

	1916 Feb. 6	10	14	18	22	26	Mar. 1	5
Observed area	129.4	111.0	105.5	99.5	67.5	57.0	45.0	37.0
Calculated "		113.0	96.8	81.6	67.9	55.9	45.5	36.6

	Mar. 9	13	17	21	25	29	Apr. 2
Observed area	27.5	23.9	17.6	14.0	11.8	9.2	5.9
Calculated "	29.2	23.1	18.1	14.0	10.8	8.26	6.27

	Apr. 6	10	14	18	22	26	30	May 4	6
Observed area	4.1	3.0	1.7	1.8	1.0	0.65	0.7		Cicatrization.
Calculated "	4.7	3.5	2.6	1.9	1.4	1.0	0.7	0.5	"

As shown by the above experiments, 0 of the formula, that is, the theoretical healing, corresponds to an area of about 0.4 sq. cm. This is required by the fact that if the calculation should be continued, 0 would be reduced to infinity. Experience has proved that the calculated number 0.4 nearly always represents complete healing of the wound.

The value of the index varies between 0.02 and 0.08. It depends on the age of the patient and the size of the wound, and is much larger when the wound is smaller and the subject younger.

In conclusion, it may be stated, that, under given conditions of asepsis and dressing, the area cicatrized in a day is directly proportional to the size of the wound, to the square root of its age, and to the relation between the rate of cicatrization and the square root of the age of the wound at the time of observation. The relation between the rate expressed in function of the total area, and the square root of the age of the wound is represented by the cicatrization index i, which acts in Formula 2 as a constant characterizing each wound.

Mechanism of the Formula.

The study of a wound in the process of healing shows that the age of the wound seems to have no action whatever on the rate of cicatrization. On the other hand, since all wounds under a certain size cicatrize at about equal rate, the part of the constant i is no longer clear, as this index is not the same for every wound. If the age of the wound is of no consequence, it should be suppressed, and the index i, which has after all the dimensions of an acceleration $\frac{V}{T}$, should tend towards the same value for all wounds when they are nearly healed. Therefore i would not be a constant characterizing every wound. How then can this index be of value in the formula as a constant? And what part does the square root of the age of the wound play?

The explanation is simple. The most important factor of the rapidity with which a wound cicatrizes is its size. Consequently when a large wound cicatrizes and grows old, the measure of the progress of cicatrization is its age. Time is one of the factors of its dimensions. Then, if age itself does not influence the rate of cicatrization, the size plays an important part. Therefore a quantity representing the age, a known quantity, represents also in some measure the size of the wound, an unknown quantity. Thus a factor inactive in it-

self, such as age, plays the part of the principal factor, that is size. But it should be understood that the area is expressed in time.

Hence the part of the index i as a constant is explained. In order to express what really occurs, i should increase in a manner inversely proportional to the size, which conforms with the formula:

$$\frac{\frac{S-S'}{S}}{t}$$

but then, the unnecessary element T must be suppressed. Practically' the coefficient i is constant, but we arbitrarily maintain the quantity T in the formula. The result is the same, because, like i, T increases in inverse proportion to the area of the wound. So the progress of cicatrization can be indirectly expressed. This procedure was used, since time was the only factor at our disposal for the calculation of the entire curve of the wound after the first two observations had been made.

Details of the Calculation.

We see from Formula 2 that in order to continue the calculation and obtain successively the area S''' at the end of t'' days, S'''' at the end of t''' days, S_n at the end of t_m days, and so on, the last calculated area is brought into the formula in order to obtain the next area. Then the following calculations are made:

$$\begin{aligned}
S'' &= S' - [S' \times i \times (t' + \sqrt{T+t'})] = S'' \text{ (here: } T = t) \\
S''' &= S'' - [S'' \times i \times (t'' + \sqrt{T+t''})] = S''' \text{ (“ } T = t + t') \\
S'''' &= S''' - [S''' \times i \times (t''' + \sqrt{T+t'''})] = S'''' \text{ (“ } T = t + t' + t'') \\
&\cdots\cdots\cdots

(2) Calculation of the surface:

(*a*) 4 days later $\begin{cases} S'' = S' - [S' \times i \times (t' + \sqrt{T + t'})] \\ = x = 9 - [9 \times 0.0416 \times (4 + \sqrt{4 + 4})] = 6.45 \text{ sq. cm.} \end{cases}$

(*b*) 3 days later $\begin{cases} S''' = S'' - [S'' \times i \times (t'' + \sqrt{T + t''})] \\ = 6.45 - [6.45 \times 0.0416 \times (3 + \sqrt{8 + 3})] = 4.84 \text{ sq. cm.} \end{cases}$

and so on.

11 days after the first observation, the area will be 4.8 sq. cm., if the wound has remained aseptic.

SUMMARY.

The cicatrization of sterile wounds may be studied in the same way as an ordinary physicochemical phenomenon. It is possible, therefore, to express the law of cicatrization by a mathematical equation as soon as an accurate measure of the wound can be obtained. By means of the equation, a curve is obtained which represents the theoretical evolution of the cicatrization of a wound. This curve, being an expression of what should happen on a normal wound, healing aseptically, on a normal man, is a daily point of comparison to what appears actually on the observed wound, and allows one to study accurately the fluctuations of cicatrization on a given individual, and the action of different dressings and antiseptic substances.

CICATRIZATION OF WOUNDS.

III. The Relation between the Age of the Patient, the Area of the Wound, and the Index of Cicatrization.

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(Received for publication, June 20, 1916.)

The observation of many wounds in the process of cicatrization, on patients of different ages, has shown that there is a proportional relation between the age of the patient, the area of the wound, and the index calculated by means of the formula

$$\frac{\frac{S - S'}{S}}{t + \sqrt{T}} = i \qquad (1)$$

In other words, one of these quantities may be considered a continuous function of the other two.

A chart has been plotted, and from the curves thus obtained, the intermediate points may be computed without calculation (Text-fig. 1). The index, the only unknown quantity, may thus be obtained directly. It then becomes unnecessary to take two measurements of the wound, 4 days apart, S and S', and one no longer needs to calculate the index i by Formula 1. On the other hand, it is evident that the index is purely theoretical, or rather an average index, which expresses normal cicatrization on a normal individual, and that marked differences may be observed between the index of a given individual, according to his general condition, and the index of the average individual of the same age.

In the course of many experiments it was found that, as a rule, the average, or normal index, was practically the same as the calculated index. Table I shows some of the figures used in making the curves.

TABLE I.

Area.	Index.	Age.	No. of patient.	Similar observations (No. of patient).
sq. cm.		*yrs.*		
130—				
129	0.0236	22	360	
125 –				
120—				
118	0.021	36	263	
115 –				
110—				
105 –	0.022	35	269	
100— 101	0.0264	21	327	
95 –				
90—				
85 –				
80—				
75 –				
70—	0.0225	27	366	
65 –				
64	0.020	32	318	
60—				
55 –				
50—				
46	0.0223	38	408	
45 –				
40—	0.0445	20	361	
35 –	0.031	33	266	
30—	0.05	21	Ja. (75)	444
25 –	0.03	38	408	383
20—				
16	0.057	27	221	289, 300, 336
15 –	0.046	37	217	408, 403, 450
12	0.068	22	256	
10—	0.065	29	289	479, 415, 366 (2 experiments).
7	0.060	31	330	
5 –	0.070	39	354	
2	0.070	30	286	409 (2 experiments).
0—				

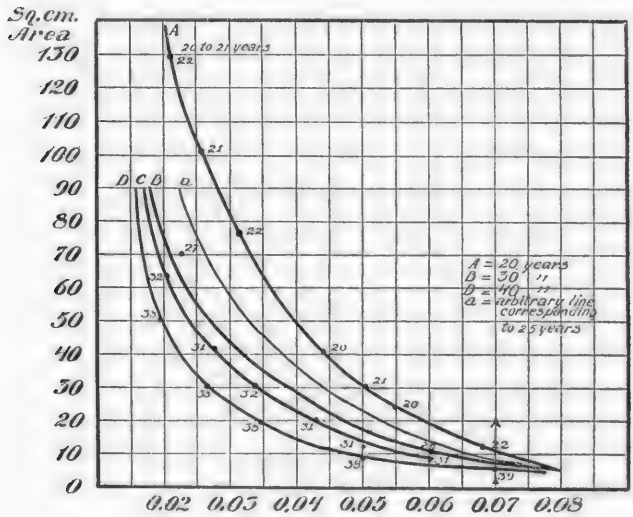
The figures show that the index is generally small for the larger wounds (about 0.020), that it increases for the smaller wounds, and that, the area of the wounds being the same, young individuals have the largest index. Since the index indicates the activity of cicatrization, the formula may be expressed:

$$\frac{ds}{dt} = f(A, i)$$

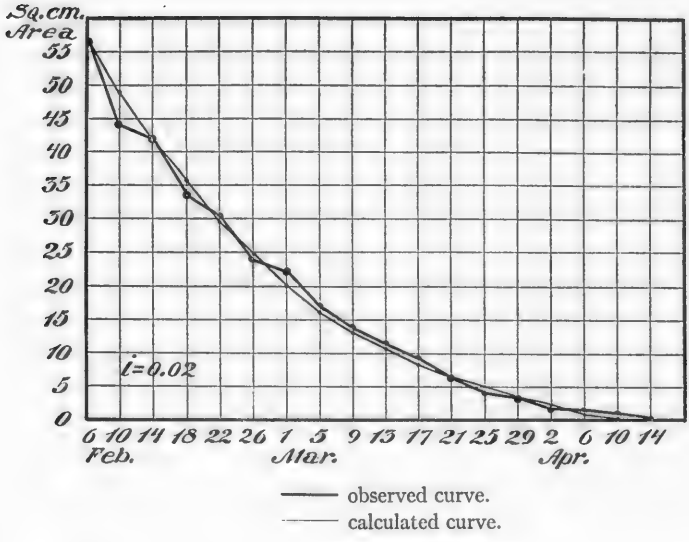
in which A is the age of the patient; S, the area of the wound; i, the index. The curves representing the ages are of the general form: $S^a \times i = K$ (K being a constant).

Text-fig. 1 has been plotted from thirty-five observations of wounds, taken at the beginning of sterilization.

The curves in Text-figs. 2 to 9 have been calculated by means of the index given in Text-fig. 1.



TEXT-FIG. 1. Composite curve for obtaining index directly.

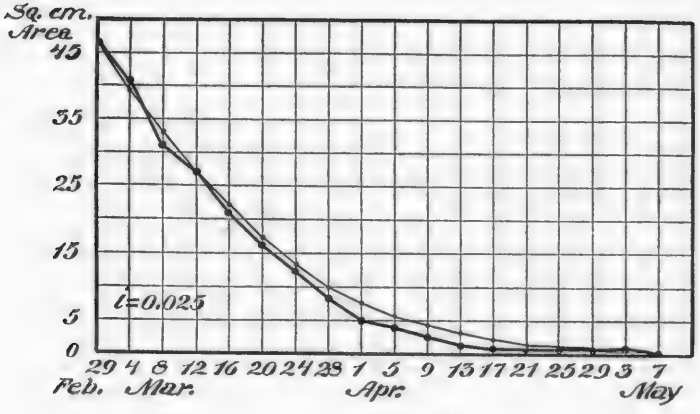


TEXT-FIG. 2.

Experiment 23.—Patient 318, age 32 years (Text-fig. 2). Index = 0.02.

	1916 Feb. 6	10	14	18	22	26	Mar. 1	5	9	13	17
Observed area.	56.6	44.3	42.0	33.6	30.2	24.2	22.1	17.0	13.6	11.0	9.2
Calculated "		49.1	42.0	35.4	29.7	24.9	20.1	16.4	13.2	10.5	8.3

	Mar. 21	25	29	Apr. 2	6	10	14
Observed area.....	6.5	4.2	3.1	1.8	1.15	0.8	Cicatrization.
Calculated "	6.6	4.3	3.1	1.8	0.70	0.2	"

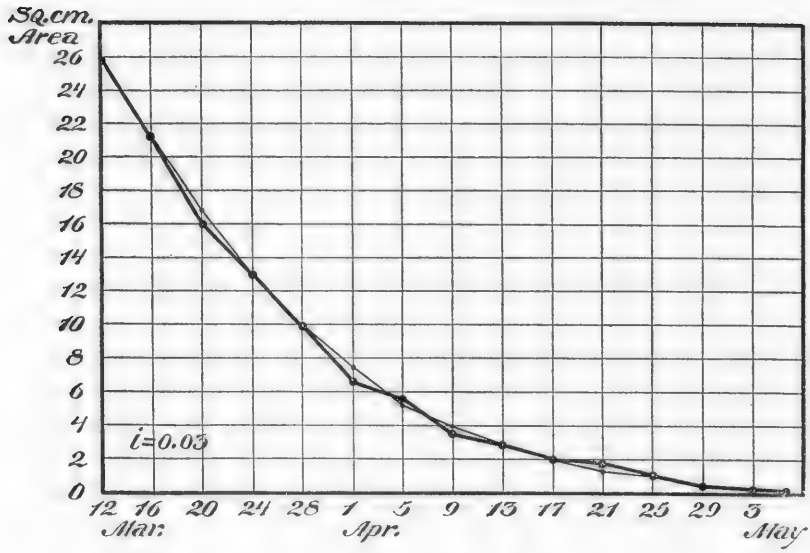


TEXT-FIG. 3.

Experiment 27.—Patient 408, age 38 years (Text-fig. 3). Wound of the thigh. Index = 0.025.

	1916 Feb. 29	Mar. 4	8	12	16	20	24	28	Apr. 1	5
Observed area.....	46.7	40.5	31.1	27.0	21.1	16.0	12.5	8.3	5.0	4.0
Calculated "		39.7	32.9	26.8	21.4	16.9	13.1	10.0	7.5	5.6

	Apr. 9	13	17	21	25	29	May 3	7
Observed area.....	2.3	1.4	1.0	1.0	0.9	0.5	0.7	Cicatrization.
Calculated "	4.2	3.1	2.2	1.6	1.1	0.8	0.6	"

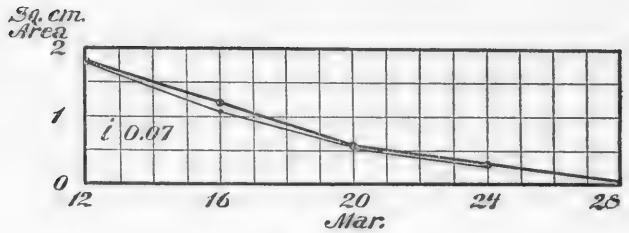


TEXT-FIG. 4.

Experiment 28.—Patient 408, age 38 years (Text-fig. 4). Wound of the arm. Index = 0.03.

	1916 Mar. 12	16	20	24	28	Apr. 1	5	9	13	17	21
Observed area.	25.8	21.1	16.0	13.0	9.9	6.5	5.6	3.6	2.7	2.0	1.7
Calculated "		21.1	16.8	13.0	9.9	7.4	5.4	3.9	2.7	1.9	1.3

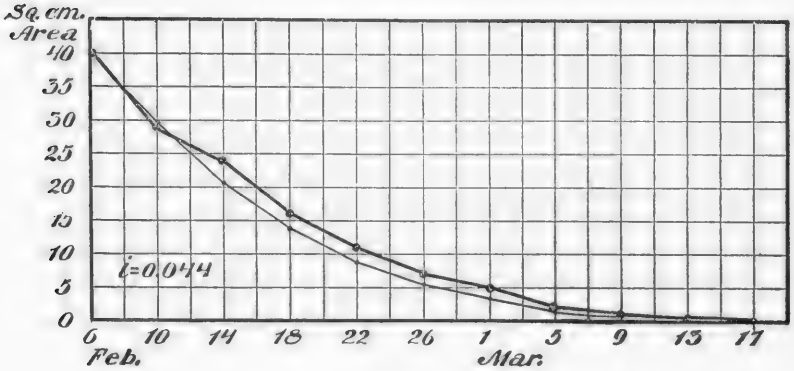
	Apr. 25	29	May 3	7	
Observed area.....	0.9	0.5	0.3	Cicatrization.	
Calculated "	0.88	0.59	0.4	"	



TEXT-FIG. 5.

Experiment 29.—Patient 408, age 38 years (Text-fig. 5). Wound of the arm. Index = 0.07.

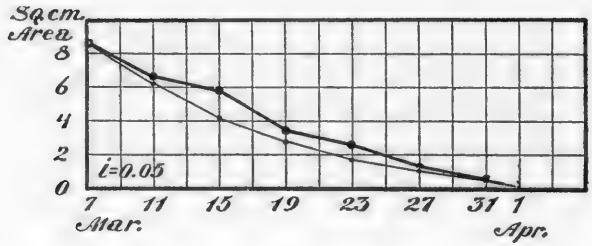
	1916 Mar. 12	16	20	24	28
Observed area.....	1.8	1.2	0.6	0.3	Cicatrization.
Calculated "		1.05	0.55	0.27	"



TEXT-FIG. 6.

Experiment 30.—Patient 361, age 20 years (Text-fig. 6). Wound of the back. Index = 0.044. Wound infected.

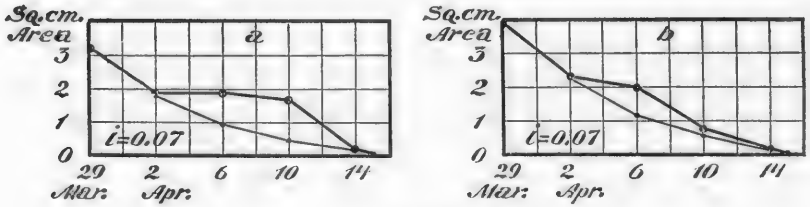
	1916 Feb. 6	10	14	18	22	26	Mar. 1	5	9	13	17
Observed area.	40.0	29.3	24.0	16.0	11.0	7.1	5.0	2.0	1.0	0.65	Cicatrization.
Calculated "		29.4	20.5	13.7	8.9	5.5	3.3	1.8	1.1	0 64	"



TEXT-FIG. 7.

Experiment 31.—Patient 403, age 40 years (Text-fig. 7). Index = 0.05.

	1916 Mar. 7	11	15	19	23	27	31	Apr. 1
Observed area.....	8.7	6.6	5.6	3.4	2.5	1.2	0.5	Cicatrization.
Calculated "		6.1	4.1	2.8	1.7	1.0	0.5	"



TEXT-FIG. 8.

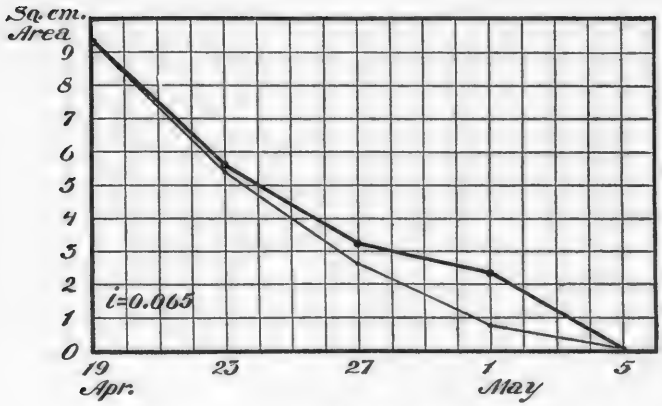
Experiments 32 and 33.—Patient 366, age 27 years (Text-fig. 8). Index = 0.07

Wound A.

	1916 Mar. 29	Apr. 2	6	10	14	15
Observed area.....	3.2	1.9	1.9	1.7	0.2	Cicatrization.
Calculated "		1.85	0.96	0.46	0.21	"

Wound B.

	1916 Mar. 29	Apr. 2	6	10	14	15
Observed area.....	3.9	2.3	2.0	0.75	0.2	Cicatrization.
Calculated "		2.25	1.2	0.56	0.25	"



TEXT-FIG. 9.

Experiment 34.—Patient 415, age 31 years (Text-fig. 9). Index = 0.065.

	1916 Apr. 19	23	27	May 1	5
Observed area.....	9.3	5.6	3.2	2.3	Cicatrization.
Calculated "		5.4	2.6	0.72	"

SUMMARY.

The first article of this series showed that it was possible to express mathematically the phenomenon of cicatrization. The principal point consisted in determining by means of an equation, a constant, or index, characterizing each wound. The calculation had to be made for each patient for each wound, and required two observations, 4 days apart.

The index having proved to be a continuous function of the size of the wound and of the age of the patient, of the form

$$S^a \times i = K$$

where S is the area, i the index, a a decimal exponent, and K a constant, it was then possible to draw a chart by means of which this index i could be obtained without calculation.

The advantage of the new way of determining the index is, above all, that this index is a general, average, normal index, and no longer an individual index. Hence, the differences between the observed

rate of cicatrization of man and the normal rate may give some indication of the general state of the patient. Another advantage is that the determination of the index is no longer controlled by the temporary accidents which may happen between the two observations of S and S'.

THE PROPHYLAXIS OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).

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(Received for publication, June 27, 1916.)

The prevention of Weil's disease can be undertaken on the basis of the excretion of the pathogenic cause in the urine and feces of patients, and on the routes of invasion of the spirochetes into the human body. But prophylaxis by active immunization seems to offer the surest method.

Prophylaxis by Active Immunization.

Before proceeding to the active immunization of the human body we made preliminary experiments on guinea pigs. The materia, to be inoculated (vaccine) was made in the following manner: To the liver emulsion or the pure culture (in Noguchi's culture medium as modified by Inada and Ido), which contained from 10 to 15 spirochetes in a single field,[1] carbolic acid was added in the proportion of 0.5 per cent, after which the mixture was left for 1 week in the ice box. The clear supernatant fluid was employed for injection into the peritoneal cavity of the guinea pigs three times at intervals of 7 to 9 days. The quantity introduced at the first injection was 2 to 3 cc., at the second 2 to 4 cc., and at the third 2 to 4 cc. The immediate effect of the injection was sometimes to cause feeble convulsions of the body, which disappeared completely in half an hour. The preservative may be responsible for this effect. The temperature often rose to 38–40°C. and continued there for 1 to 2 days. In some instances the guinea pigs, as a result of repeated inoculations, lost weight and appetite, and died. From 9 to 18 days after the last inoculation, 2 to 3 cc. of a liver emulsion or of the pure culture con-

[1] $\frac{1}{12}$ oil immersion, oc. 3 (Leitz), under dark-field illumination.

TABLE I.

Animal No.	Date and quantity of serum inoculated.			Injection of spirochetes.		Result.	Day of disease of control animals.	Result of Pfeiffer's test with blood serum of experimental animals.
	I	II	III	Date.	Quantity.			
1	May 26 2 cc.	June 1 2 cc.	June 9 2 cc.	June 26	Liver emulsion 2 cc.	Died on same day.		
2	"	"	"	" 24	"	Condition good until 23rd day. Killed.	Died of icterus on 5th day.	Positive.
3	May 1 2 cc.	May 24 2 cc.	May 30 2 cc.	" 17	"	Died on following day. Autopsy findings negative.		
4	June 26 2 cc.	July 4 2 cc.	July 10 4 cc.	July 16	"	Died on 8th day. No icterus. Autopsy findings negative.		
5	July 4 2 cc.	July 10 4 cc.	July 16 4 cc.	" 26	Pure culture 2 cc.	Condition good until 19th day. Killed.	Died of icterus on 5th day.	Positive.
6	"	"	"	"	"	"	"	"
7	July 8 2 cc.	July 14 4 cc.	July 22 4 cc.	Aug. 1	Pure culture 3 cc.	Died of dysentery on 14th day. Autopsy findings negative.	"	

8	July 8 2 cc.	July 14 3 cc.	July 22 4 cc.	Aug. 1	Pure culture 3 cc.	Condition good until 17th day. Killed. Autopsy findings negative.	Died of icterus on 5th day.	Positive.
9	"	"	"	"	"	"	"	
10	"	4 cc.		"	"	"	"	
11	July 10 3 cc.	July 16 4 cc.	July 22 4 cc.	"	"	"	"	
12	"	"	"	"	"	Died of dysentery on 14th day. Autopsy findings negative.	"	
13	"	"	"	"	"	Condition good until 17th day. Killed. Autopsy findings negative.	"	
14	"	"	"	"	"	"	"	Positive.
15	"	"	"	"	"	Died on 3rd day. Autopsy findings negative.	"	

taining 6 to 15 spirochetes in a field were injected into the peritoneal cavity. Table I gives the result of this experiment. 9 of the 15 animals survived for 17 days or longer. 6 died of diarrhea, 1 on the day of injection, 1 the day after, 1 on the 3rd day, 1 on the 8th, and 2 on the 14th day. The autopsy findings were negative. They showed neither icterus nor hemorrhage, and no spirochetes were found in the liver or kidneys under dark-field illumination. The livers of the guinea pigs that died on the 8th and 14th days were emulsified and injected intraperitoneally into the other guinea pigs. Of these animals only the first died of the typical disease, while the others remained healthy. That the spirochetes used for the infection were highly virulent was shown by the control animals, which, having been inoculated at the same time with an equal quantity of pure culture, succumbed after 5 days to the typical disease. Hence it was shown that guinea pigs are highly susceptible to Weil's disease and can be immunized actively by inoculations of a vaccine containing the killed spirochetes or their disintegration products.

In order to ascertain the fate of fresh virulent spirochetes injected intraperitoneally in immunized guinea pigs, we examined every hour the peritoneal fluid of the nine animals mentioned above. 30 minutes after the injection, the spirochetes were few and the majority already degenerated; after 2 hours either no specimen was found or very few; after 10 to 24 hours none were found. We killed the nine guinea pigs and examined them for immune bodies in the blood serum and for spirochetes in the liver and kidneys. The blood serum was positive by Pfeiffer's test, and no spirochetes were found in the liver or kidneys on dark-field illumination. From these experiments it is apparent that the spirochetes gradually disappear in from a few to 24 hours from the peritoneal cavity of the actively immunized guinea pigs; and yet that sometimes virulent spirochetes may still be present after 3 days in the liver and also in the kidneys.

It is, however, remarkable that the blood serum does not contain the immune bodies in demonstrable quantity, even after three inoculations of the vaccine, with Pfeiffer's test, although the guinea pig is protected from infection by virulent spirochetes. However, on injection of the fresh spirochetes the immune bodies appear in the blood in quantities demonstrable by Pfeiffer's test. We shall have something to add on this point later.

Briefly, we could show that spirochetocidal and spirochetolytic bodies develop in the blood of the guinea pig after repeated inoculations which protect the animal from an injection of fresh, virulent spirochetes. We have not yet tested the duration of this active immunity.

Passive immunization is perhaps not possible. We injected as much as 10 cc. of the immune serum into guinea pigs, which was followed after several days by inoculation of active spirochetes. All the animals developed typical infections. No protection was given. But when immune serum and spirochetes were injected at the same time, no infection took place; the guinea pigs were protected from the disease.

Prophylaxis through Protective Inoculation in Man.

We undertook the immunization of a horse through inoculation with vaccine. After demonstrating the appearance of immune bodies in the blood of the horse, we proceeded to the active immunization of man.

At first we tested the vaccine which was employed for immunizing the guinea pigs, but unsuccessfully. Later we prepared a vaccine ten times as strong as the first and with it obtained good results for the first time. Individuals subject to, but who had not had Weil's disease were inoculated subcutaneously, and tests were made for the immune bodies after a definite period.

The body temperature of each person was taken for 1 week. The blood was taken from a vein, and the serum kept in the ice box. The inoculations were made at intervals of 5 days, at the first injection 0.5 cc., at the second 1 cc., and at the third 2 cc. being given. The blood was drawn 10 days after the last injection. Pfeiffer's tests were made with the serum taken before and after the vaccine injection, and with them a control test with isotonic salt solution was made also. The results of the tests are shown in Table II.

From the table it is seen that Pfeiffer's test made with the serum taken before inoculation does not differ from the control test, while that made with the serum drawn after the inoculation shows the presence of immune bodies.

TABLE II.

Experiment No.	Animal No.	Inoculated into peritoneal cavity of guinea pigs.*		No. of spirochetes in peritoneal fluid.		Result.	Autopsy findings.	Spirochetes in liver.
		Serum.	Spirochetes.	After 30 min.	After 2 hrs.			
Control.		Isotonic salt solution 1 cc.	Pure culture (10 in 1 f.) 1 cc.	2–3 in 1 f.	2–3 in 1 f.	Died of icterus on 4th day.	+++	+++
1	1	K. G. serum before inoculation 1 cc.	"	"	1–2 in 1 f.	Died of icterus on 6th day.	+++	+++
	2	K. G. serum after inoculation 1 cc.	"	1–2 in 1 l.	1 in one specimen.	Died on 13th day. No icterus.	+	+
2	3	E.G. serum before inoculation 1 cc.	"	2–3 in 1 f.	1 in 1 f.	Died of icterus on 4th day.	+++	+++
	4	E.G. serum after inoculation 1 cc.	"	2–3 in 1 l.	1 in 1 l.	Died of icterus on 8th day.	++	++
3	5	F.T. serum before inoculation 1 cc.	"	2–3 in 1 f.	1 in 1 f.	Died of icterus on 6th day.	+++	+++
	6	F.T. serum after inoculation 1 cc.	"	1 in 3 f.	1 in 2 l.	Died of icterus on 10th day.	++	++
4	7	N. Z. serum before inoculation 1 cc.	"	2–3 in 1 f.	1 in 2 f.	Died of icterus on 4th day.	+++	+++
	8	N. Z. serum after inoculation 1 cc.	"	None in one specimen.	None in one specimen.	Died of icterus on 12th day.	+	++
Control.		Isotonic salt solution 1 cc.	Liver emulsion (10 in 1 f.) 1 cc.	7–8 in 1 f.	6–7 in 1 f.	Died of icterus on 4th day.	+++	+++
5	9	M. S. serum before inoculation 1 cc.	"	6–8 in 1 f.	5–6 in 1 f.	Died of icterus on 4th day.	+++	+++
	10	M. S. serum after inoculation 1 cc.	"	None in one specimen.	None in one specimen.	Died of icterus on 8th day.	+++	+

Control.		Isotonic salt solution 1 cc.	Pure culture (10 in 1 f.) 1 cc.	2–3 in 1 f.	2–3 in 1 f.	Died of icterus on 3rd day.	++	+++
6	11	I. H. serum before inoculation 1 cc.	"	1 in 1 f.	2–3 in 1 l.	Died of icterus on 6th day.	+++	+++
	12	I. H. serum after inoculation 1 cc.	"	1–2 in 1 l.	1 in a few l.	Died of icterus on 9th day.	++	+
Control.		Isotonic salt solution 1 cc.	Liver emulsion (10 in 1 f.) 1 cc.	1–2 in 1 f.	1–2 in 1 f.	Died of icterus on 6th day.	+++	+++
7	13	M. K. serum before inoculation 1 cc.	"	"	"	Died of icterus on 5th day.	+++	+++
	14	M. K. serum after inoculation 1 cc.	"	2 in a few l.	1 in one specimen.	Died of icterus on 10th day.	++	++
8	15	K. S. serum before inoculation 1 cc.	"	1 in 1 f.	1 in 1 f.	Died of icterus on 6th day.	+++	+++
	16	K. S. serum after inoculation 1 cc.	"	1 in one specimen.	None in one specimen.	Died of icterus on 11th day.	++	+
9	17	A. K. serum before inoculation 1 cc.	"	1–2 in 1 f.	1 in 1 f.	Died of icterus on 5th day.	+++	+++
	18	A. K. serum after inoculation 1 cc.	"	None in one specimen.	None in one specimen.	Died on 15th day. No icterus.	+	+

*The spirochetes were always searched for with dark-field illumination ($\frac{1}{12}$ oil immersion, oc. 3, Leitz).
f. = one field.
l. = from one side to the other of the cover glass (65 to 70 fields).

In the first instance one sees that as regards Pfeiffer's test the spirochetes in the peritoneal fluid are identical with the serum taken before the inoculation and the salt solution; while the blood serum taken after the inoculation yields after 30 minutes scarcely any spirochetes, and after 2 hours merely a degenerate specimen in a preparation. The guinea pig died, however, after 13 days, without icterus; that is, 7 to 9 days later than the control animal and the animal with serum taken before the inoculation. The internal organs showed some hemorrhage and a few spirochetes in the liver. Hence the existence of immune bodies in the blood is shown, although still insufficient to prevent all infection. The ninth animal gave a similar result. The other animals did not show as high a grade of immunity as these two. The difference in survival between the immunized and the control animals amounts in several cases, respectively, to 7, 8, and 10 days, in 2 cases to 5, in 3 cases to 4, and in 1 case to 3 days. The difference of 4 days indicates the lowest limit of the incomplete immunity. If the serum contains no immune bodies, as is the case in the blood serum of normal individuals, the experimental animal succumbs to the typical disease on the same day as the control, or at the most, a day or two earlier or later. A difference of 3 days is seldom seen. If, therefore, the experimental animal dies of the typical disease 4 days later than the control animal, we may conclude that the blood serum of this animal contains a certain quantity of immune bodies. The greater the quantity of immune bodies in the blood serum, the longer is the time of incubation for the guinea pig, and finally when complete immunity is attained, the animal no longer becomes ill after injections. This phenomenon of gradually increasing immunity can readily be followed by Pfeiffer's test of the blood drawn each day after the 6th or 7th day of the disease. This experiment thus indicates that the immune bodies appear in the blood after inoculation.

The question arises as to whether or not this incomplete immunity is able to protect the organism against infection with *Spirochæta icterohæmorrhagiæ*. As we are not in a position to decide this question by the injection of the pathogenic cause directly into man, we are obliged to draw our conclusions indirectly from the immunity reaction of patients and from animal experimentation.

This state of incomplete immunity is observed in patients after the 8th to the 11th day of the disease. In this stage (the second stage according to Inada and Ido, or the icteric period according to Oguro) the experiments of infection with the blood of the patient are, as a rule, negative. The spirochetes in the blood have all been killed by the immune bodies. After the 8th or 9th day of the disease, there are likewise no, or very few degenerated spirochetes in the liver. The spirochetes in the liver have already been destroyed by the immune bodies in the incomplete state of immunity. It is not possible to demonstrate immune bodies by Pfeiffer's test within a week after the onset of the disease. The experimental animals die as rapidly as the controls. Nevertheless, one recognizes from the infection experiments conducted with the blood of patients, that a certain quantity of the immune bodies must have appeared in the blood. The intraperitoneal injection of 2 or 3 cc. of blood into the guinea pig produces the disease in typical form up to the 4th day in 100 per cent of cases (26 cases, all positive), on the 5th day in 91.6 per cent (only 1 case among 12 being negative), on the 6th day in 85.7 per cent (only 2 negative cases among 14), on the 7th day in 50 per cent (4 negative and 4 positive cases). These gradually decreasing percentages of positive results can be explained only by the appearance of the immune bodies. The almost completely negative findings in the liver on the 7th day of the disease indicate the appearance of immune bodies even inside of 1 week. Nevertheless, up to the 7th day we cannot prove the existence of immune bodies in the blood serum by Pfeiffer's test. But, we may conclude that the degree of partial immunity described above suffices to protect the organism against infection from the spirochete of Weil's disease.

By means of these animal experiments it was possible to establish the fact that three inoculations protect the guinea pig against infection with the spirochete; but notwithstanding this active immunity, it is not possible to demonstrate by Pfeiffer's test immune bodies in the blood serum of the guinea pig (Table III).

We inoculated six guinea pigs, giving each three injections of 1 cc. of vaccine after an interval of 5 days, and then injected intraperitoneally into three guinea pigs pure cultures of *Spirochæta ictero-*

TABLE III.

Animal No.	Date and quantity inoculated. I	II	III	Spirochetes injected.	Result.	Autopsy findings.	Spirochetes in liver.	Result.
1	Jan. 20 1 cc.	Jan. 25 1 cc.	Jan. 29 1 cc.	Pure culture (30 in 1 f.) 1 cc.	Died of dysentery on 19th day.	−	−	−
2	"	"	"	"	Died of dysentery on 16th day.	−	−	−
3	"	"	"	"	Died of dysentery on 18th day.	−	−	−
Control.				"	Died of icterus on 4th day.	+	+	+
1	Jan. 22 1 cc.	Jan. 27 1 cc.	Jan. 31 1 cc.	Not injected.	Killed Feb. 9 to get blood serum.			
2	"	"	"	"	"			
3	"	"	"	"	"			

Pfeiffer's test.

Animal No.	Inoculated into peritoneal cavity of guinea pigs. Serum.	Spirochetes.	No. of spirochetes in peritoneal fluid. After 30 min.	After 2 hrs.	Result.	Autopsy findings.	Spirochetes in liver.	Result.
1	V_1 serum 1 cc.	Pure culture (10 in 1 f.) 1 cc.	2–3 in 1 f.	2–3 in 1 f.	Died of icterus on 5th day.	+++	+++	−
2	V_2 serum 1 cc.	"	"	1 in 1 f.	"	+++	+++	−
3	V_3 serum 1 cc.	"	"	2 in 1 f.	Died of icterus on 4th day.	+++	+++	−
4	Isotonic salt solution 1 cc.	"	"	2–3 in 1 f.	"	+++	+++	−

hæmorrhagiæ. Three other animals were killed and their serum was tested by Pfeiffer's method. The three guinea pigs into which the pure cultures were injected died of diarrhea after 16 to 19 days, without showing the typical disease. The autopsy findings were completely negative in all. No spirochetes could be found in the organs. 4 days later, the control animal died of the typical disease. In the serum of the three guinea pigs that were killed no trace of immune bodies could be demonstrated by Pfeiffer's test. The experimental animals died of icterus and hemorrhage as soon as the control animal. From these experiments it is evident that subsequent to the inoculation, the serum contains immune bodies, but while the small number makes it impossible to prove the existence of the organism by Pfeiffer's test, it is sufficient to prohibit a proliferation of the spirochetes in the body of the guinea pig. After the injection of virulent spirochetes into the inoculated guinea pig, the immune bodies appear in the blood. From the clinical findings and the animal experiments we may conclude that a quantity of the immune bodies in the blood, which cannot be proved by Pfeiffer's test, acts prophylactically against infection with *Spirochæta icterohæmorrhagiæ*.

The secondary effects of the inoculation are scarcely noticeable in man. In the guinea pig we often observed fever following an injection, but this never occurred in man. In two cases there was slight headache and general lassitude, but these manifestations were negligible and disappeared completely in from 1 to 3 days. The chief complaint was of pains at the site of injection. One often sees local swellings and slight redness, but these disappear after 24 hours. The pains also are not severe.

It is not known how long the partial immunity continues, but it is probable that it lasts from 6 months to a year.

DISCUSSION AND SUMMARY.

We have already described briefly the portals of entry and of excretion of the pathogenic spirochetes.[2] We may mention here

[2] Inada, R., Ido, Y. Hoki, R., Kaneko, R., Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

that we have twice prevented epidemics by disinfection of the ground and the removal of the inundated water in certain places in coal mines. In one mine 19 out of 50 workmen, and in another 9 out of 30 workmen came down with Weil's disease in about 2 weeks.

We have already pointed out that the period during which the pathogenic spirochetes are excreted in the urine continues, as a rule, for 40 days, and that we must, therefore, apply disinfection for at least 40 days after the first appearance of the disease. Lately we have found that in 21 cases out of 24 the spirochetes were excreted in the urine for 40 days, in one case until the 42nd day, in one case until the 45th day, and in still another case until the 63rd day.

Another important fact concerning the prophylaxis which has been brought out is that both house and ditch rats (brown) carry virulent *Spirochæta icterohæmorrhagiæ*, the causal spirochete of Weil's disease, in their kidneys. Miyajima[3] has reported that field rats have the pathogenic organisms in their kidneys; he will report these findings in detail later. The spirochetes which he described are less virulent than ours. On his advice we have carefully examined house and ditch rats in the city and rats in the coal mines of Kyushu, where Weil's disease prevails, and found that 39.5 per cent carried highly virulent pathogenic spirochetes in their kidneys, thus confirming Miyajima's experiments. The kidneys were examined microscopically under the dark-field microscope, and in the cases in which we did not find the pathogenic spirochete, we made inoculations into guinea pigs. Thus we found *Spirochæta icterohæmorrhagiæ* microscopically in the kidneys or in the urine in 32.4 per cent, and by means of inoculation in 7 per cent, making a total of 39.5 per cent carrying the pathogenic organisms, out of a total number of 86 rats examined. In some instances, rats were made to bite guinea pigs and in two instances caused Weil's disease. Among fifty-five patients in our clinic, twelve were cooks; and in Europe many cases arise among butchers—indicating the relation of the disease to rats. Moreover, during the present year we observed two patients who acquired Weil's disease, one in 1 week, the other 8 to 9 days after they had been bitten by rats. These facts point to a relation be-

[3] Reported at the April, 1916, meeting of the Fellows of the Kitasato Institute for Infectious Diseases.

tween Weil's disease and rats. The infection is transmitted probably from rats to man by means of the urine of the rats, directly or indirectly. On the injection of 0.1 gm. of rat urine which contains *Spirochæta icterohæmorrhagiæ* into the peritoneal cavity of guinea pigs, the infection arises, while the injection of the liver or the blood of the rats into guinea pigs does not produce the typical disease.

The finding that the kidneys of rats contain the pathogenic organisms of the disease is important from the point of view of prophylaxis. The large number of rats in the trenches of the European battle-fields suggests the possibility that many cases of Weil's disease may arise. We shall report on this point in more detail later.

THE SERUM TREATMENT OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).

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Although Weil's disease has been recognized since antiquity and its mortality has been high, treatment up to the present time has been purely symptomatic, as the causative agent was not known. But with the discovery of the cause, we have now entered upon the study of the specific therapy. In the course of their discovery of the causative agent, Inada and Ido[1] found in the blood serum obtained from recovered cases of the disease an immune body which acted as a solvent and destroyed the spirochetes contained in the blood and the liver of the guinea pigs under experimentation; on the basis of this work they advanced the hypothesis that serum therapy should be effective in Weil's disease.

In his experimental investigation Ido obtained remarkable results with convalescent serum and active serum from immunized goats. He demonstrated that the immune serum, if injected prior to the appearance of icterus, is capable of inhibiting the disease in all cases. This work forms the basis of the serum therapy of Weil's disease in man. On the other hand, experiments undertaken with salvarsan, as already indicated elsewhere,[2] were not followed by comparable results. For these reasons we have been led to employ antiserum in the treatment of Weil's disease since August, 1915; and we were in a position to report the results of our work as early as October, 1915.

[1] Inada and Ido, A Report on the Discovery of the Causal Organism (a New Species of Spirochæta) of Weil's Disease, *Tokyo Ijishinshi*, 1915, No. 1908.

[2] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., The Etiology, Mode of Infection, and Specific Therapy of Weil's Disease (Spirochætosis Icterohæmorrhagica), *J. Exp. Med.*, 1916, xxiii, 377.

Up to the present time serum therapy has not been widely employed in the spirochetal diseases. Since the serum therapy of readily relapsing spirilloses leads to the formation of a serum-fast strain, it has been claimed that salvarsan would be more suitable for treatment in these cases. But in Weil's disease a serum-fast strain does not occur. Spirochetolytic and spirochetocidal immune bodies, however, develop in the blood, and the immunity lasts for several years, the antibodies having in one case been unmistakably detected 8 years after recovery. Relapse apparently does not occur in Weil's disease. We have never found a case of it, although Fiedler recognizes it. Inada and Ido have given the term "after fever" to the fever which occurs on the 14th or 15th day from the onset of the disease, sometimes on the 13th to the 16th day, less frequently on the 12th to the 17th, and which continues for several days, often from 7 to 10. But this cannot be considered a relapse in the real sense of the word, because at this stage of the disease we do not find the spirochetes in the blood, or in any organs but the kidneys.

We employed first serum from recovered cases and later serum obtained from actively immunized horses. As human convalescent serum is not procurable in large quantities, a horse was immunized with *Spirochæta icterohæmorrhagiæ*. The goat also can be readily immunized, but is too small an animal to yield large amounts of serum. At the present time we possess two actively immune horses, which are able to take an injection of 800 cc. of pure culture, containing 30 spirochetes per field, under dark-field illumination ($\frac{1}{12}$ oil immersion, No. 3 ocular, Leitz).

The serum which we now have is far more potent than that obtained at the beginning. 0.01 cc. of the present serum injected simultaneously with a pure culture into the peritoneal cavity protects a guinea pig weighing 200 gm. against infection from 1 cc. of pure culture (10 spirochetes per field). In the treatment of the patients considered here, the serum was of weaker concentration, as much as 0.5 cc. of the serum being required to protect a guinea pig against infection from a similar dose of culture. Inasmuch as our most potent serum was obtained only toward the end of 1915, the opportunity to test its action did not arise, as we have had no cases of Weil's disease since that time.

To the serum obtained from horses, 0.5 per cent carbolic acid was added and it was kept in the ice box for 1 month. It was then tested for tetanus toxin and carbolic acid content, and also by cultures, to establish sterility. We employed serum taken on July 12, August 28, September 19, and October 11 from Horse 1. 35 patients in all received the treatment; 23 in the clinic and 12 cases occurring in a coal mine. Of these patients, 6 were treated with human convalescent serum, 2 with both horse and human serum, and the remaining 27 patients with horse serum alone. As the titer of the horse serum about equalled that of the human serum, the cases treated will be discussed jointly.

Dose of Serum Injected.—The initial daily dose for 3 days was 10 cc., but our experience derived from a number of cases shows that this dosage is ineffectual. For that reason we employed gradually larger doses, until finally we injected 60 cc. in 24 hours. This quantity of serum was given in three amounts, with intervals of from 5 to 6 hours. Of late, we have made intravenous injections with good results. It was feared in the beginning that the toxin liberated by lysis of the spirochetes would prove harmful to the organism, but this fear proved to be unfounded.

The day on which serum treatment was instituted is shown in Table I.

TABLE I.

Day of illness.	No. of cases.
2	4
3	4
4	5
5	6
6	8
7	3
8	5

In 24 of the cases icterus was present at the beginning of the serum treatment; 11 cases showed no icterus when the treatment was begun; and in 5 there was no icterus during the course of the disease. As the serum, according to our experience, is mainly spirochetolytic and spirochetocidal in action, and its antitoxic effect has not yet been

demonstrated, it is recommended that it should be employed as early as possible after the onset of the disease,—if possible, before the 5th day of illness, when there are probably many spirochetes present in the organs. The distribution from the point of intensity of the disease was as follows: 17 severe cases, 17 moderately severe, and 1 mild case.

The criteria employed to determine the result of the serum therapy were:

1. The rate of mortality.
2. The influence of the serum upon the spirochetes in the circulating blood.
3. The effect of the serum on an earlier development of antibodies.
4. The number of spirochetes found in the organs after death, when serum treatment had been employed.
5. The influence upon the symptoms and the progress of the disease.

Of these criteria, special emphasis has been placed upon Nos. 2 and 3, inasmuch as they afford definite objective phenomena for judging the effect of the serum treatment, and are valid even if the number of patients is small, while for the other criteria a large number of observations is required. The difficulty of determining the effect upon the symptoms and the course of the disease in individual cases increases with an early use of the serum, as in that case it is not easy to decide whether the slight illness is attributable to the serum, or whether the disease as such was of a mild character. The prognosis in most cases is determined at the height of illness, or on the 7th or 8th day from the onset.

Mortality in Weil's Disease.

Of the 35 patients who received the serum treatment, 7 died. Of this number, 2 showed complications, 1 developed suppurative meningitis on the 27th day of the disease, and another patient who had a suppurative process died after 2 months. This leaves only 5 cases in which the cause of death is attributable to Weil's disease itself rather than to a complication. Of these, one patient, who was admitted in a moribund condition and died on the following day, has not been included. Omitting these cases, the mortality was found to be 11.4 per cent. If we exclude also the 12 patients in the coal mine and include only the 23 cases treated at the clinic, the mortality is 17.3 per cent.

According to Oguro, the mortality of Weil's disease in Japan is 40 per cent, according to Nishi, 48 per cent; and according to Inada, 30.6 per cent. As we are not in possession of definite data regarding the death rate among the patients in the coal mine, we have excluded these cases and have compared only the mortality of patients in our clinic, treated with serum or otherwise, with the general statistics. The mortality in our clinic up to last year was 30.6 per cent. With the beginning of the serum therapy, it will be seen that the mortality is reduced almost to two-thirds. It must, however, be stated that we have, during the past year and since the discovery of the causative agent, accepted a relatively large number of patients, and among these there were probably a number who had the disease in a mild form.

In view of the reduction in the mortality rate, at the moment, we shall limit our claim to the statement that the administration of the serum has a marked effect in Weil's disease, and we shall await the results obtained from a large number of cases before making a more positive assertion.

The Behavior of Spirochæta icterohæmorrhagiæ in the Circulating Blood after Serum Injection.

We desire to present in brief form the result of infection experiments carried out with the blood of patients who received no serum treatment. Table II shows the result of this investigation which comprises 68 cases, arranged according to the day of illness.

TABLE II.

Infection Experiments with Blood from Weil's Disease.

Day of illness.	No. of cases.	Results.		Per cent of positive results.
		Negative.	Positive.	
Up to 4	26		26	100.0
5	12	1	11	91.6
6	14	2	12	85.7
7	8	4	4	50.0
8	4	4	0	0
9	1		1	100.0
12	1	1		0
18	1	1		0
19	1	1		0

It will be seen that in all cases up to the 4th day of illness, the result was positive; of the experiments undertaken on the 5th day 91.6 per cent were positive; on the 6th day, 85.7 per cent; on the 7th, 50 per cent. From the 8th day on, of blood taken from 8 patients, only one, that taken on the 9th day, proved to be positive.

In order to determine whether the dose of serum employed is capable of killing and dissolving the spirochetes in the circulating blood of the patient, blood was taken, before the injection of serum, from the arm vein of the patient and injected intraperitoneally into guinea pigs; and, similarly, another sample of blood was drawn after the injection of the serum. If, then, the guinea pig receiving the blood drawn before the injection of serum became typically ill, while that receiving the blood drawn after serum had been injected into the patient remained well, the last drawing of blood having been made on the 4th day of the disease, we may conclude that, in the latter case, the spirochetes in the circulating blood had been destroyed by the serum. When the blood is taken on the 5th or 6th day, the determination is somewhat more difficult, inasmuch as blood injected on the 5th day gave negative results in 8.4 per cent, and that drawn on the 6th day was negative in 14.3 per cent of the cases. It is possible to estimate the spirochetocidal action of the serum from the difference in the percentages of infectability of the injected blood (Table III).

TABLE III.

Infection Experiments with Blood Drawn from Patients with Weil's Disease before and after the Injection of Serum into the Patient.

No.	Day of illness on which serum treatment was given.	Results.		Quantity of serum injected.	Time between serum injection and drawing of blood.	Day of disease after serum treatment.	Positive results.	
		Before serum injection.	After serum injection.				With serum treatment.	Without serum treatment.
				cc.	*hrs.*		*per cent*	*per cent*
1	2	+	+	10	30	3		
2	2	+	−	40	6	3	25.0	100.0
3	2	+	−	60	24	4		
4	2	+	−	40	24	4		
5	2	+	−	60	24	5		
6	3	+	+	20	24	5		
7	3	+	±	60	24	5		
8	3	+	−	60	24	5		
9	4	+	+	20	5	5	50.0	100.0
10	4	+	+	10	7	5		
11	4	+	−	80	12	5		
12	5	+	−	20	5	5		
13	3	+	±	40	4th day.	6		
14	4	+	−	35	22	6		
15	5	+	−	40	13	6		
16	5	+	+	10	7	6		
17	5	+	+	10	24	6	71.4	77.7
18	5	−	−	60	17	6		
19	5	+	+	60	12	6		
20	6	+	+	10	3	6		
21	6	−	−	10	7	6		
22	6	+	−	10	12	7		
23	6	+	±	40	15	7	33.3	100.0
24	6	+	−	80	7	7		
25	7			60				
26	8			60			0	0
27	8	−	−	10	7	8		

Table III shows that in four cases in which human blood not subjected to serum treatment was capable of infecting guinea pigs, when drawn on the 4th day of the disease after serum treatment had been given, the infection was positive in only a single case. The

ratio of infection of the guinea pigs with blood drawn after the administration of serum is, therefore, 25 per cent. If we compare this figure with the percentages of infection (100 per cent) found in guinea pigs receiving blood taken before serum treatment, a vast difference is apparent. This difference in results justifies us fully in concluding that the blood taken from patients after they have received serum treatment is no longer capable of infecting guinea pigs. This means either that the serum has destroyed the spirochetes in the circulating blood, or that the spirochetes have lost their virulence and are no longer capable of producing an infection. The positive result in a single case is to be attributed to the insufficient dosage of the serum, only 10 cc. having been injected in the case in question. On the 5th day the percentage of guinea pigs infected after serum treatment was 50 per cent; without serum treatment, 100 per cent. The positive result in 3 cases is also due to the insufficient quantity of serum administered, as only from 10 to 20 cc. had been injected. In one case the infection experiment proved negative even when 20 cc. of the serum had been administered.

On the 6th day the percentage of illness of the guinea pigs, subsequent to serum treatment, was 71.4; without serum treatment, 77.7. Among the patients there was one who received 60 cc. of the serum, and whose blood was still capable of infecting guinea pigs after a period of 20 hours. It is difficult to say why the spirochetes in the blood were not destroyed by the serum.

On the 7th day of illness, 33.3 per cent of guinea pigs infected with blood taken after serum treatment became ill, and 100 per cent without serum treatment. Here we have the blood of a patient which caused an infection in one guinea pig, while another animal remained well. This case has been included among the positive results.

From these results we may conclude that the horse serum which we have prepared, administered in suitable doses, is capable of destroying completely the spirochetes contained in the circulating blood, by the injection of 40 to 60 cc. of serum in from 5 to 6 and up to 24 hours. A quantity of 10 to 20 cc. of the serum did not suffice to kill the spirochetes.

The Influence of the Serum Treatment upon the Appearance of Antibodies in the Blood.

As we have stated elsewhere,[3] the antibodies in Weil's disease are not demonstrable by Pfeiffer's method in the blood serum of patients inside of 1 week from the onset of illness. Beginning with the 8th or the 11th day, antibodies make their appearance in the blood, although they are at first weak; by the 12th or the 13th day they are fully active. They are rarely active as early as the 8th day. The time of development of the antibodies in the blood seems to be an individual characteristic, and differs according to the severity of the disease. We have designated as incomplete, with respect to antibody formation, those cases in which the infected animals (tested by Pfeiffer's method) outlived the control animals by 4 days or longer. A difference of 3 days in the life of the experimental and the control animals should be regarded as an indication of incomplete immunity, but we have taken 4 days as a conservative limit.

TABLE IV.

The Period of Development of Antibodies in the Blood of Patients with Weil's Disease.

Day of illness.	No serum treatment.				Serum treatment.			
	Complete development.	Incomplete development.	Negative.	Total.	Complete development.	Incomplete development.	Negative.	Total.
6			3	3	1	1	7	9
7			5	5		2	3	5
8	2	2	6	10	3	8	7	18
9		3	4	7	3	1	2	6
10		1	2	3	5	5	2	12
11	1		2	3		1	1	2
12	3		1	4	3			3
13	43			43	7			7

One patient treated with the serum showed complete development of antibodies in the blood on the 6th day of illness. This phenomenon never occurs with the blood of patients who have not received the serum. As shown in Table IV, in a large percentage of the patients

[3] Ido, Y., Hoki, R., Ito, H, Wani, H., The Prophylaxis of Weil's Disease (Spirochætosis Icterohæmorrhagica), *J. Exp. Med.*, 1916, xxiv, 471.

receiving serum treatment complete development of antibodies could be demonstrated as early as the 9th or 10th day, while this condition appeared only on the 11th or 12th day when no serum had been administered. If we also include the cases of incomplete development of antibodies, we are justified in concluding that when serum treatment is given, the antibodies make their appearance in the blood earlier than in those cases where no serum is administered. As far as the dosage of serum in relation to the development of antibodies is concerned, this is difficult to estimate, for the intensity of the disease and the individual circumstances must always be taken into account, but speaking generally it may be said that development proceeds more rapidly with a large dose of serum.

The Influence of Serum Treatment upon the Spirochetes in the Body.

As Kaneko and Okuda[4] have discussed this point in detail, we shall present only a brief outline here. It may be said, in general, that the number of spirochetes found at autopsy in the bodies of patients who had received serum treatment is much less than that found in patients ill for an equal period of time who received no serum; and, moreover, the spirochetes in the first instance show a high degree of degeneration. This statement is particularly true of the kidneys and the cardiac muscles.

The Influence of Serum Treatment upon the Symptoms and the Course of the Disease.

Whether or not the serum has an appreciable effect upon the fever, icterus, and hemorrhagic diatheses can be determined only by more extensive experience. Up to the present time, we have observed no significant effect, except that with serum treatment the hemorrhages are less frequent than without serum treatment. The "after fever," on the other hand, seems to occur more frequently when serum has been given. Serum therapy has no definite effect upon the excretion of spirochetes in the urine, although the period of excretion of the

[4] Kaneko and Okuda, The Pathological and Anatomical State of the Lymph Glands in the Early Stage, and the Distribution of the Spirochæta in Them, *Tokyo Ijishinshi*, 1915, No. 1940–1945.

living spirochetes seems to be shorter where large doses of serum have been employed.

Secondary Manifestations.

We have observed few secondary effects. Following the injection of serum, no fever occurred. In one case only was there a complaint of headache, in another of tinnitus, and in still another there appeared an exanthem at the site of injection. These symptoms which were all mild in character disappeared completely after 1 or 2 days. Frequently complaint was made of slight pains at the site of injection, but these also disappeared on the following day.

Spirochetocidal and Spirochetolytic Action of Serum.

We have recently observed a case in which we were able to demonstrate experimentally upon guinea pigs in a conclusive manner the spirochetocidal and spirochetolytic action of serum from Weil's disease. The patient was a man of 65, who was taken ill suddenly on May 1. He was admitted to the clinic on the 4th day of the disease. A large number of *Spirochæta icterohæmorrhagiæ* were found in the blood under dark-field illumination; *i.e.*, 14 to 16 on a cover slip (60 to 70 optical fields). In no other case have we found the spirochetes so numerous, for as a rule the organisms are not readily found in fresh blood preparations. The number of spirochetes found before and after serum injection was as follows:

May 4, 2.00 p.m. Admitted to clinic, 4th day of illness. Spirochetes in the blood, 14 to 16 per field, as described above.
3.00 p.m. 17 cc. of serum, very active (from Feb. 26), subcutaneously.
6.00 p.m. Spirochetes 14 to 16.
7.00 p.m. Spirochetes 10 to 16.
8.00 p.m. 20 cc. of serum, intravenously.
10.00 p.m. No spirochetes in 2 preparations.
12.00 m. No spirochetes in 3 preparations.
1.30 a.m. No spirochetes in 1 preparation.
At 1.00 a.m. we injected 2 cc. of the blood of the patient into the peritoneal cavity of a guinea pig. Up to the time of writing, May 14, the animal has remained well. At 8.30 a.m., 3 cc. of the blood were injected into the peritoneal cavity of another guinea pig. This animal also has not become ill.

2 hours after intravenous injection, we were unable to find any spirochetes. This result in the experimental animal is in entire accord with the spirochetocidal and spirochetolytic action of the serum observed in human beings.

SUMMARY.

Horses immunized with cultures of *Spirochæta icterohæmorrhagiæ* yield an immune serum having therapeutic properties.

With rare exceptions the serum destroys completely the spirochetes contained in the circulating blood.

The development of antibodies is promoted by the serum injections.

The number of spirochetes in the organs is reduced by the treatment.

Secondary manifestations due to the serum are slight and disappear promptly.

The ultimate effects of the serum treatment on the symptoms and final outcome of the disease have still to be determined.

EXPERIMENTAL OBSERVATIONS ON THE PATHOGENESIS OF GALL-BLADDER INFECTIONS IN TYPHOID, CHOLERA, AND DYSENTERY.

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The fact that the chronic carrier in the typhoid group of diseases is a result of infection is becoming of more and more importance in epidemiology. In most cases the microorganisms are known to be carried in the gall-bladder or gall-passages. In cholera, the infection seems to produce nearly the same result. At least semichronic intestinal carriers have been found and their occurrence has been shown by Kulescha (1), Greig (2), Schöbl (3), and others to be due to infection of the gall-bladder. This discovery is the most important recent addition to the pathology and epidemiology of the disease. In the case of bacillary dysentery, fewer cases have been studied from this point of view, but intestinal carriers have been reported, and in a few instances dysentery bacilli have been recovered from the bile (4), and analogy and experiment suggest a similar set of circumstances. In short, in this whole group of diseases, one of the most important problems of preventive medicine seems to be the prevention and cure of gall-bladder and gall-passage infections.

The subject has been approached from the experimental side by making use of gall-bladder infections in the rabbit. In some respects, at least, the lesion in the rabbit is a counterpart of that in man, and it seems probable that, in working out means of preventing and curing carriers, the experimental lesion in the rabbit will be an important factor. The present paper deals with experimental observations on the mechanism of gall-bladder infection in typhoid, concerning which our knowledge at present is still uncertain; the mechan-

ism of infection in cholera and dysentery by a portal system septicemia is suggested, and the antiseptic properties of rabbit bile are emphasized.

Mechanism of Gall-Bladder Infection in Typhoid.

Logically, as has often been stated, the gall-bladder lesion might be due to descending infection of the bile from the liver, to an ascending infection of the bile from the intestines, or to a transverse infection through the gall-bladder wall from the blood-vessels. The theory of descending infection from the liver was most generally accepted up to 1908 when Koch (5) and Chiarolanza (6) claimed to have proved that transverse infection through the gall-bladder wall is the usual method. Their views are more or less accepted at present (7). The theory of ascending infection from the intestines was early abandoned on account of the rarity of clinical gall-bladder infections with *Bacillus coli* and on account of positive results following intravenous injections in animals with or without ligation of the common duct.

It is desirable to settle this question if possible, because from the point of view of therapeutic attack, at least for prophylaxis, it makes a great deal of difference whether the infection occurs from the bile or from the blood vessels of the bladder wall. The writer is convinced that Koch and Chiarolanza's work is not conclusive and believes that we should go back to the theory of descending infection from the liver as an explanation of the usual method of infection. Occasionally, of course, infection might occur in any one of the three ways, but it seems reasonable that there is some regular method.

HISTORICAL.

The observations on which the theory of descending infections rests date back to our early knowledge of the elimination of microorganisms by the liver. Apparently the first work done was that of Fütterer (8), who, in 1888, in connection with the study of cases of typhoid with cholecystitis, reported that he injected a culture of *pyocyaneus* into the left ventricle of rabbits and recovered the organism in the gall-bladder at least 1½ hours later. In 1895 Fütterer (9) reported that he made a gall-bladder fistula in a dog and recovered *Staphylococcus aureus* from the bile 40 minutes after subcutaneous injection. He em-

phasized the importance of the liver in freeing the blood from microorganisms, and also referred to bacilli in the gall-bladder as a possible cause of relapse in typhoid, but of course, at that time, the possibilities of chronic cholecystitis in the spread of the disease were not realized. In 1899 Fütterer (10) recorded further experiments with dogs in which he established a common duct fistula and gave a portal vein injection of a suspension of *Bacillus prodigiosus* and recovered the organism from the bile 2 minutes after injection.

The work of Fütterer was confirmed by several workers, especially by Biedl and Kraus (11) who, in 1896, took up the question of elimination of bacteria by the bile in the course of a general study of the elimination of microorganisms by the liver, kidneys, and salivary glands. They made common bile-duct fistulas in dogs and recovered staphylococci after intravenous injection in 13 to 45 minutes. In 1905 Doerr (12) made a more particular study of the typhoid gall-bladder problem and in investigating the method of infection tied the cystic duct in rabbits; after several days he gave an intravenous injection but obtained no infection. However, when he ligated the common duct and gave an intravenous injection, an infection of the gall-bladder resulted.

These observations established the excretory powers of the liver and seemed to afford a natural explanation for gall-bladder infections in a septicemia like typhoid. In 1908, however, Koch and Chiarolanza published their articles in which they claimed to have demonstrated that infections occur through the gall-bladder wall and not through the bile. Koch drew his conclusions from the histological picture in a human case of typhoid cholecystitis in which he found emboli of bacilli in the folds of the mucous membrane. Chiarolanza tied the cystic duct and immediately injected typhoid bacilli intravenously and recovered them from the gall-bladder. He concluded that the usual method of infection was through the gall-bladder wall. However, as will be shown later, his experiments form a slender basis for adopting this theory, although many recent articles assume that the gall-bladder wall infection method is the proved method of infection (7).

Technique.

A number of experiments have been made by the fistula method. At first large Belgian hares, weighing 10 kilos, were used in order to make the operation easier, but it was found that smaller animals, even guinea pigs, can be easily used. Under ether anesthesia, a median incision is made from the ensiform cartilage to below the umbilicus, the stomach and duodenum are drawn down by an assistant, putting the common duct on the stretch; the mesentery over the duct is cut, avoiding the vessels, and a threaded aneurysm needle is passed under the duct about 1 inch from the duodenum. The thread serves

to cut off the bile while the capillary tube is being inserted and to tie the tube in place. The thread is put on the stretch by an assistant to constrict the duct, a small transverse incision is made below the constriction with a small pair of scissors, the capillary tube, which rapidly fills with bile, is inserted, the thread is tied on the neck of the tube, the rubber tubing attachment is brought out by a small opening in the right side of the abdominal wall, and the wound is closed. The rubber tube is attached to a glass tube in a two-holed stopper and the stopper is inserted in a graduated centrifuge tube. Bile soon begins to drop and accumulates at a rate of 1 cc. every 5 to 8 minutes, depending on the size of the animal. The capillary and rubber tube hold about 0.5 cc. of bile. If care is used in inserting the tube and in tying the duct securely, no blood enters the duct and brilliant green bile can be obtained in large quantities. With proper asepsis few contaminations are found in the plated bile. After injection, the bile is plated in 0.5 and 1.0 cc. quantities, and the colonies are counted after 48 hours' incubation. Most of the typhoid, cholera, and dysentery colonies were typical, but in case of doubt, they were identified by staining, by the double sugar tube, and by agglutination. Mesenteric injections were made into the veins of the appendix; some bleeding occurs when the needle is withdrawn, but it stops spontaneously and no gangrene of the intestine follows.

Most of the animals were about the same weight (3 kilos). The time of collection of bile was limited to about 1 hour, as it was found that cultures taken after 1 hour up to 24 hours were almost always sterile, even when many bacilli came through in the 1st hour.

The writer realizes, of course, that bacilli may appear in the bile from foci in the liver after the 1st hour, but the short observation time brings out points of importance and there is a limit to the experimental method on account of emaciation of the animal and infection of the field of operation. Moreover, the process of elimination seems to resemble that of filtration, and Bull (13) has shown that the blood after intravenous injection is usually sterile in less than an hour. Most of the animals were chloroformed in 1 or 2 days but two animals lived over a week before they were killed. Altogether twenty-four rabbits and two guinea pigs were successfully operated on.

EXPERIMENTAL.

If the theory of descending infection is accepted as a working hypothesis and we attempt to test it experimentally it is evident that two conditions must be met; first, the bacilli must get into the bile, and second, they must be able to multiply in the bile. Infection of the rabbit's gall-bladder with typhoid bacilli has been produced many times by intravenous injection since the first work on this subject by Blachstein (14) and Welch (15) in 1891, but the results are uncertain and on an average not more than 50 per cent of inoculations are successful. In order to secure 100 per cent of infections which are necessary in therapeutic tests many authors have used the direct inoculation of the gall-bladder. This method is almost always successful, but it introduces a new element of traumatism and escape of blood into the gall-bladder and throws no light on the ordinary method of infection. If an explanation of the failure of intravenous injections to produce 100 per cent of infections can be found it may furnish a clue to the solution of the whole problem.

Presence of Bacilli in the Bile.—In the first set of experiments, the cystic duct was tied in order to prevent the possible entrance of organisms from the gall-bladder; a common duct fistula was then made, which, incidentally, prevented any organisms coming from below, and an injection was made into a mesenteric vein in order to favor concentration of the organisms in the liver. 1 cc. of a 24 hour broth culture was used for injection; the bile was collected every 5 to 15 minutes and poured into mannite media or over the surface of a double sugar tube, or Loeffler's media, depending on the organism used. In this way I obtained positive evidence of the presence in first specimens of bile of typhoid, paratyphoid A, and dysentery Y bacilli, and cholera vibrios. These experiments convinced me that microorganisms regularly enter the bile from the liver if they are present in the blood in sufficient numbers. In the rest of the experiments the cystic duct was not tied and the bile was plated in order to obtain quantitative results.

Relation of the Number of Bacilli Injected to the Number of Bacilli Appearing in the Bile.—It is known that, in general, larger intravenous doses produce more positive results in the gall-bladder and

several experiments have been carried out to determine what dose is necessary for bacilli to appear in the bile. Fistulas were established and different doses of a 24 hour broth culture of a recently isolated typhoid bacillus were injected into the ear vein. Bile was collected and plated, and the colonies were counted after 48 hours' incubation (Table I).

TABLE I.

Number of Colonies in Plated Bile after Injection into Ear Vein of Different Quantities of Typhoid Bacilli.

Bile.	1 cc.	2 cc.	3 cc.
cc.			
0.5	—	—	—
0.5	—	—	50
0.5	—	—	25
0.5	—	—	6
0.5	—	—	10
1.0	—	1	4
1.0	—	—	2
1.0	—	—	—
1.0	—	—	3
1.0	—	—	2
Total colonies in 7.5 cc. of bile.....	0	1	102
Time of collection in min.....	65	45	45

These experiments show that a fairly large dose is necessary for bacilli to appear in the bile in any number after ear vein injections, and may explain why some injections fail to produce lesions. There is probably some individual variation in elimination, but four other rabbits given 1 cc. and one other rabbit given 2 cc. failed to show any colonies, so the figures given above may be taken as fairly typical.

When bacilli appear, the first plates show the largest number of colonies; as the capillary and rubber tube hold about 0.5 cc. it is evident that the bacilli begin to appear in the bile very quickly and can be demonstrated in collected bile after 2 or 3 minutes. If a large enough dose is given, bacilli will therefore appear in the bile, but they may not be able to multiply and produce a lesion as will appear later when the antiseptic action of rabbit bile is considered.

Relation of the Place of Injection to the Number of Bacilli Appearing in the Bile.—Further evidence along this line is furnished by injection into the mesenteric veins. If gall-bladder infections are due to bacilli in the bile it should be possible to produce more infections by mesenteric than by ear vein injections and more bacilli should appear in the bile in the former instance. Such is the case; after intravenous injection into mesenteric veins seven out of eleven rabbits, or 63 per cent, had gall-bladder lesions at the end of a week, while of twelve controls given the same dose into an ear vein, only five, or 41 per cent, showed a lesion. The number of bacilli appearing in the bile after mesenteric vein injection was larger than after ear vein injection, although there was more variation than in the ear vein injection experiments given above. 1 cc. of a 24 hour broth culture injected into the portal system resulted in thirty-one colonies in the plated bile as compared with none in the control experiment, but in a second experiment no colonies appeared. Similarly, in the case of cholera vibrios, 1 cc. produced twenty-eight colonies in one instance and none in the control, but in a second experiment no colonies appeared. In general, however, on the basis of considerable experience with the two methods, I can state that more organisms appear in the bile after mesenteric vein injection than after ear vein injection. The evident bearing of this fact on production of gall-bladder lesions in cholera and dysentery will be discussed later.

Relation of Immune Animals to the Number of Bacilli Appearing in the Bile.—Evidence is also furnished by the observation of Dr. K. F. Meyer, soon to be published, that immune animals show a somewhat higher percentage of infections of the gall-bladder than normal animals. This condition has been investigated by the same technique mentioned above with results shown in Table II. Meyer immunized the animal with three intravenous injections. Its agglutination was strong at 1:10,000. The test dose was 1 cc. of a 24 hour broth culture with one control not immunized.

TABLE II.

Number of Colonies in Plated Bile in an Immunized Animal and in a Normal Animal.

Bile.	Normal animal.	Immunized animal, agglutination, 1:10,000.
cc.		
0.5	—	—
0.5	—	50
0.5	—	90
0.5	—	45
0.5	—	11
1.0	—	20
1.0	—	—
1.0	—	1
1.0	—	—
1.0	—	1
Total	0	218
Time of collection in min	50	50

In this experiment the bacilli in the immunized animal came through in large numbers, while the control showed none. In two other vaccinated animals with an agglutination of only 1: 6,000, only a few colonies appeared. These results can be best explained in accordance with the recent work of Bull (13) by the rapid agglutination *in vivo*, the deposition of bacilli in the liver, and corresponding elimination. In other words, gall-bladder infections in vaccinated animals are not necessarily an index of lack of immunity, but may, in part, be an indication of a rich amount of immune bodies in the blood. Greig (16) has reported the occurrence of gall-bladder lesions in rabbits highly immunized with cholera-like vibrios. These results confirm my previous contention (17) that the gall-bladder lesion in the rabbit cannot be used to test general immunity from vaccination, and conclusions based on gall-bladder infections in immunized animals must be revised with this fact in mind.

The first condition necessary to confirm the descending infection theory, namely, the presence of bacilli in the bile, is fulfilled by the work stated above; more bacilli appear with larger doses, more bacilli appear with mesenteric vein injection than with ear vein in-

jection, and more bacilli appear in immunized animals. In regard to the theory of infection through the gall-bladder wall, on a critical examination of the work of Chiarolanza who tied the cystic duct and secured infection after intravenous injection, it is found that his protocols state that the bile is bloody even in animals examined in 24 hours, and he obtained 100 per cent of infections of the gall-bladder with the cystic duct tied and only 74 per cent of infections among normal animals.

Chiarolanza apparently assumed that the bloody appearance of the bile was due entirely to infection. As a control on this experiment, I tied the cystic duct of two rabbits but gave no injection; the next day the animals were chloroformed and the bile in the gall-bladder was examined. It had a reddish color and contained desquamated epithelium. It appears, therefore, that in Chiarolanza's work the bile was simply a diluted blood culture, as in tying the cystic duct the cystic artery is also tied and hemorrhagic infarction occurs from collateral circulation. As has been mentioned above, Doerr performed the same experiment of tying the cystic duct, but waited several days before giving an intravenous injection, and under these conditions no infection occurred. In other words, the bladder wall had recovered to such an extent that no blood and consequently no bacilli entered the gall-bladder. Doerr's experiments are therefore much more conclusive than Chiarolanza's and they support the descending infection theory.

The mechanism of infection in paratyphoid infections is in all probability the same as that in typhoid. Carriers are well known in paratyphoid A (18) and B (19, 20) infections, and acute and chronic cholecystitis has been described in both cases. In the rabbit Doerr (12) has reported persistent gall-bladder lesions after intravenous injections of both organisms. In four of fifteen animals I have obtained gall-bladder lesions with intravenous injections of a recently isolated paratyphoid A bacillus.

Gall-Bladder Infection in Cholera and Dysentery as a Result of Portal Septicemia.—As has been shown above, the evidence points to descending infection of the bile from the liver as the cause of gall-bladder infection in a septicemia like typhoid.

In cholera, Schöbl, favors the ascending route from the duodenum and cites the vomiting of cholera vibrios as evidence that they may be present in the duodenum and stomach. Greig (2) refers to a lymphatic infection, but it is not clear how the lymph reaches the gall-bladder from the intestines. In a later article (21) Greig apparently also accepts the direct infection of the gall-bladder wall. Kulescha favors the theory of descending infection on account of foci of infection found in the liver. While cholera is not usually classed as a septicemia and cholera organisms have been recovered from the blood rarely, Greig (2) reports their presence in the lungs, and they have been found in the urine a number of times (22).

These facts point to an occasional invasion of the blood and it seems likely that if blood cultures could be made from the mesenteric veins positive results would be more frequently obtained. Experimental evidence favors this mechanism of gall-bladder infection as it has been shown above that more organisms appear in the bile after mesenteric vein injection than after ear vein injection. The gradual development of immunity would also favor the deposition of organisms in the liver and their increased elimination by the bile.

In dysentery, blood cultures are occasionally successful (4) and dysentery bacilli have been found in various organs, including the gall-bladder, at autopsy. In cholera and dysentery it seems likely, therefore, that gall-bladder infections are due to a portal or general septicemia with elimination of organisms in the bile as in typhoid. The occurrence of liver abscess in amebic dysentery is suggestive along the same line.

In experimental animals persistent gall-bladder lesions have apparently not been produced by intravenous injections of cholera vibrios. A considerable amount of work has been done with animals by Greig (21), Cano (22), Schöbl (23), Baroni and Ceaparu (24), and others, but in each case in which the vibrios have been found in the bile after intravenous injection the animals died or were killed in a few hours. In cases of survival after injection, Schöbl found no lesions in guinea pigs, and Greig found no lesions in one rabbit.

A possible explanation of the failure in guinea pigs will appear later. In three rabbits given ear vein injections of 1 cc. of a 24 hour broth culture, I found no lesions and no vibrios in the bile at the end of 1 week. In three rabbits given the same dose by a mesenteric vein, one animal had bloody bile, but no vibrios were found in it. Further work on the rabbit with more careful dosage is indicated.

Direct inoculation of the gall-bladder of the guinea pig was uniformly successful in Schöbl's hands, and in the rabbit I have produced three persistent lesions in three trials.

With dysentery bacilli less work has been done. Doerr (12) found the Flexner type in the bile 24 hours after intravenous injection in the rabbit, but not after 4 and 14 days. With the Y type I have produced three lesions after four trials by mesenteric vein injection and one after three trials by ear vein injection. By direct inoculation of the Flexner type, out of five trials, five lesions were present at the end of a week. Further experimental work with intravenous injections is needed. Cholera vibrios and dysentery bacilli may be less able to produce gall-bladder lesions in the rabbit than the organisms of the typhoid and colon groups. The clinical percentage of chronic carriers in cholera and dysentery is apparently less than that in the typhoid group; but this may in part be due to a small percentage of cases with invasion of the blood.

Antiseptic Properties of Rabbit Bile.

The second condition to be determined in testing our working hypothesis, namely, multiplication of the organisms in the bile, evidently depends on the qualities of bile as a culture medium. It seems to be generally assumed that ox bile is a standard of bile and that bile is an excellent culture medium for the organisms of the typhoid group. As a matter of fact both these conceptions are more or less incorrect. There is no standard bile, as bile from different animals differs in its action on microorganisms. Even ox bile and human bile may have a distinctly bactericidal action on the typhoid bacillus, as has been shown by Corrado (25), Talma (26), Pies (27), Fornet (28), Meyerstein (29), and others. It is only by the addition of organic matter such as blood or pus that bile regularly becomes a good culture medium. A suggestive recent finding in this connection is that the lactose bile is inferior to the plain lactose broth for the detection of colon bacilli in water (30, 31).

In the following work the difference between the bile in the gall-bladder and the bile directly from the liver has not been considered, but this may be of some importance as the former is known to be richer in solids and, as Okada (32) has shown, may be less alkaline.

In the case of the rabbit and guinea pig the antiseptic action is strongly marked, as may be seen in Tables III and IV. One loopful of a broth culture of different organisms was put in 1 cc. of different kinds of bile. One loopful was plated immediately and one loopful was plated after 24 hours.

TABLE III.

Antiseptic Action of Bile.

1 loop plated immediately.

Bile.	Typhoid.	Para-A.	Para-B.	Cholera.	Dysentery (Flexner).	Colon.
Ox	240	320	160	10	160	640
Human	1,000	1,600	1,600	50	400	1,600
Rabbit	400	500	800	40	500	In.*
Guinea pig	400	400	500	5	640	800

* In. indicates innumerable.

TABLE IV.

Antiseptic Action of Bile.

1 loop plated after 24 hours' incubation.

Bile.	Typhoid.	Para-A.	Para-B.	Cholera.	Dysentery (Flexner).	Colon.
Ox	In.	In.	In.	In.	In.	In.
Human	"	"	"	"	"	"
Rabbit	—	—	—	—	—	—
Guinea pig	—	—	—	—	—	—

This experiment shows that typhoid, cholera, and dysentery organisms grow well in human or ox bile, but are killed after 24 hours in guinea pig and rabbit bile. All specimens of guinea pig bile killed all the organisms tested. In the rabbit there was some variation especially in regard to cholera vibrios; human and ox bile also vary in effect, but the above result is typical of a certain number of specimens. A fairly long exposure is necessary as there is not evidence of antiseptic action at the end of 1 hour. These facts are plainly of considerable importance in many ways. They may explain failure of infection even with doses large enough to insure the passage of

bacilli through the bile. They also furnish a concrete instance of a difference between an experimental animal and man. As a side issue an example may be cited of failure to appreciate this difference. Greig (21) in endeavoring to find some means of preventing cholera carriers, gave rabbits urotropin and tested their bile for vibricidal properties. As a control he used ox bile and while the vibrios grew well in ox bile they failed to grow in the treated rabbit bile. Greig notes the unusual fact that urotropin seemed to be active in an alkaline medium. It is clear from the above results that the vibrios might not have grown in a control of normal rabbit bile.

The reaction of bile from different species varies as much as does the color, and the reaction seems to be of considerable importance in explaining its action on microorganisms. Table V shows the reaction to phenolphthalein and lacmoid; a satisfactory, simple method (32) of titrating the reaction of all specimens of bile has not been found on account of the difference in color. Phenolphthalein is a good indicator for rabbit and guinea pig bile, but it is difficult to use on human and ox bile. With lacmoid the end-point is also difficult to determine exactly.

TABLE V.

Reaction of Bile.

Bile.	Color.	Phenolphthalein.	Lacmoid.
Ox	Dull brown.	Acid.	2*
Human	Golden "	"	Neutral.
Rabbit	Bright green.	Neutral.	3–6
Guinea pig	" yellow.	1 per cent alkaline.	8

* The figures for lacmoid show the number of cc. of N/1 acid required to neutralize 100 cc.

These analyses taken together with the bactericidal tests given above suggest that the alkalinity is responsible for the antiseptic action; guinea pig bile, for example, is 2 per cent more alkaline than usual culture media. If guinea pig or rabbit bile is neutralized by hydrochloric acid or sulphuric acid, the antiseptic properties disappear, as is seen in Tables VI and VII, in which the same technique was employed.

TABLE VI.

Antiseptic Action of Fresh Bile.

	Rabbit.		Guinea pig.	
	1	2	1	2
	1 loop plated immediately.	1 loop plated after 24 hours.	1 loop plated immediately.	1 loop plated after 24 hours.
Typhoid	50	—	400	—
Para-A	240	—	400	—
Cholera	10	1,600	5	—
Colon	800	—	640	—
Dysentery	480	—	800	—

TABLE VII.

Absence of Antiseptic Action of Bile When Neutralized.

	Rabbit.		Guinea pig.	
	1	2	1	2
	1 loop plated immediately.	1 loop plated after 24 hours.	1 loop plated immediately.	1 loop plated after 24 hours.
Typhoid	200	In.	800	In.
Para-A	200	"	500	"
Cholera	75	"	15	"
Colon	750	"	400	"
Dysentery	480	"	480	100

The alkalinity of the bile may not be the only factor concerned in its inhibitory action, but it must be an important factor in view of these results. It is instructive to note that of all the organisms, the only one which grew in fresh alkaline bile was the cholera vibrio which is known to grow best in an alkaline culture medium.

Prophylaxis.

These facts naturally suggest that an alkaline therapy might be of value in preventing or curing carriers. The reaction of rabbit bile can be influenced by injections of alkalies. A common duct fistula was made and 20 cc. of a 5 per cent solution of sodium bicarbonate, or 1 gm., were given intravenously and the reaction of the bile was

tested at different times. At the end of an hour the reaction was nearly twice as alkaline as at first and then it fell again.

In order to make a preliminary test of alkaline therapy in preventing gall-bladder infections, four animals were given 1 gm. of sodium bicarbonate in 5 per cent solution, intravenously, and after 1 hour they, with four controls, were given 2 cc. of a suspension of typhoid bacilli, equal to one 24 hour agar slant. At the end of a week they were chloroformed and examined; the controls showed 50 per cent of lesions, the treated animals showed 25 per cent of lesions. In other words, some prophylactic effect was apparently demonstrable. Further work is necessary but the practical suggestion for the trial of alkalies in the prevention and treatment of carriers is obvious.

It is known that gall-bladder infections in the rabbit tend to spontaneous cure, and further work on this subject should be done with a view to determine the part played by the bile in this process.

DISCUSSION.

The results of the experiments recorded above support the theory of descending infection of the gall-bladder through the bile from the liver. Infection of the gall-bladder wall cannot be absolutely ruled out and probably occurs at times, but the bile-ducts seem to be the regular avenue of infection. This conclusion suggests that prophylactic measures and possibly curative measures should be directed toward the bile rather than toward the blood stream and tissues. Vaccination, for example, appears to have little effect in the prevention and cure of experimental or clinical lesions and in fact may favor the production of lesions by increased elimination of organisms in the bile. A great deal of experimental work has been done with various drugs and synthetic substances, but the subject of the natural defences of the bile and the possibility of increasing them has been neglected. Human bile must have some antiseptic action, because, in any septicemia, some microorganisms undoubtedly pass through the bile-ducts and gall-bladder, but in only a comparatively few cases do they produce a definite cholecystitis. *In vitro*, some specimens of human bile are bactericidal or inhibitive, while others are not. Experiments into the nature of this inhibition with a view to increasing it have been done with the rabbit.

Rabbit bile is more alkaline than human bile and is more antiseptic for all the organisms considered in this paper, except in some instances for cholera vibrios. Rabbit bile has some effect in preventing lesions, because doses large enough to insure the passage of organisms through the bile and large enough to make an animal sick or even kill it often show no organisms on culture of the bile. Gall-bladder lesions in the rabbit are probably due, first, to a sufficient dose to insure a considerable number of microorganisms in the bile; second, to a low margin of the inhibitory action in the bile or to an increased resistance on the part of the strain employed; and third, to an injury of the liver cells or mucous membrane of the gall-passages and bladder with desquamation of cells and oozing of serum. Pies (27) showed that *in vitro* the inhibitory action of the bile could be abolished by the addition of serum or pus. In the direct inoculation of the gall-bladder this factor probably is important in the almost invariable success of the method. In man the production of gall-bladder lesions probably depends on the same factors as in the rabbit. The difference between rabbit and human bile is only a question of degree, and valuable lessons may be learned from the rabbit, especially in regard to the cause of failure of intravenous injections to produce 100 per cent of lesions.

It is a debatable question whether a patient suffering from an acute infection should be subjected to additional treatment in order to prevent him from the possibility of being a danger to others, provided he recovers, but if a mild therapy were of any value it would be justified. In cholera, for example, alkaline therapy is of value in preventing uremia, which is the greatest danger to the individual, next to collapse (33). If this treatment also helped to keep the bile clean, this fact should be kept in mind. However, as the cholera vibrio grows best in an alkaline medium, this line of treatment may favor cholecystitis. These questions are subjects for further investigation. When the individual recovers from an acute attack, the best practice requires that he shall not be discharged as cured until the carrier question is considered. Aside from the examination of the stool, the use of Einhorn's duodenal tube (34) seems to be a valuable aid in determining the condition of the bile.

SUMMARY.

1. The theory of the production of gall-bladder lesions in typhoid, by descending infection of the bile from the liver receives support from investigations with the common duct fistula method in the rabbit.

More bacilli appear in the bile with increased doses and more gall-bladder infections are obtained by increased doses.

More bacilli appear in the bile after mesenteric vein injection than after ear vein injection and more lesions result under the first condition.

More bacilli appear in the bile after injection of the same dose in immunized animals than in normal animals and more lesions also result in immunized animals.

In cholera and dysentery the same mechanism is suggested with the additional factor of a portal system septicemia.

2. After the appearance of microorganisms in rabbit bile, their fate is apparently largely determined by the antiseptic properties of the bile.

100 per cent infections cannot be secured by intravenous doses large enough to insure the presence of microorganisms in the bile.

Rabbit bile *in vitro* may be antiseptic to the microorganisms considered.

The antiseptic action is largely due to its alkalinity.

It is apparently possible to protect the rabbit to some degree against gall-bladder infection by a previous injection of sodium bicarbonate.

3. Alkaline therapy is suggested in the prevention and cure of gall-bladder carriers.

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STUDIES ON THE BLOOD PROTEINS.

I. The Serum Globulins in Bacterial Infection and Immunity.

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INTRODUCTION.

For a number of years much study has been devoted to the origin and the chemical nature of the antibodies which may develop within the organism during the course of an infection, or which may be elaborated within it by the various methods of immunization. The efforts to establish the chemical identity of antibodies have naturally been centered about a study of the possible relationship subsisting between the proteins of the blood and the immune bodies demonstrable in it by various serologic tests. A great stimulus to these investigations has come from the discovery of new methods of separating and of chemically identifying the different fractions which go to make up the blood proteins. Of these additions to our knowledge the method, introduced by the Hofmeister school, of separating the various protein constituents by fractional precipitation with different salts has, perhaps, produced the most far reaching results.

For some time it has been a well established fact that diphtheria antitoxin, for instance, is precipitable from serum by any precipitants which throw down the globulins. The early observations of Brodie (1), Seng (2), and Hiss and Atkinson (3) have been confirmed and extended by a number of later workers (4).

Considerable work has been done also to establish the chemical nature of bacterial antibodies. Probably one of the earliest contributions to this subject was made by Pfeiffer and Proskauer (5), who separated cholera immune serum into its globulin and albumin fractions, and showed that the cholera immune bodies which give rise to Pfeiffer's phenomenon are present only in the globulin fraction. This important study was later amplified by the classical experiments of Pick (6), who demonstrated conclusively that cholera and typhoid agglutinins also occur in one or another of the globulin fractions depending upon the species

of animal employed for immunization and the nature of the antibody studied. To these observations should be added those of Rodhain (7) and of Moll (8). According to the work of Rodhain, the immune bodies of antistreptococcus serum occur in the euglobulin fraction; and according to Moll, the development of precipitins following immunization with a foreign protein is also associated with a rise in the serum globulins of the immunized animal.

The above observations have been variously interpreted by different investigators. Whereas some workers strongly incline to the view that the antibodies in question are a form of blood globulin, others entertain the possibility that the antibody, by analogy with bacterial poisons, enzymes, and similar bodies, is mechanically carried down by the precipitate of globulin.

We believed that the only satisfactory method of procuring reliable data on the globulin-antibody problem was to make quantitative estimations of the immune bodies and of the blood proteins, not at random periods during the experiment, but at frequent and well timed intervals during the process of immunization. In this way alone is it possible to determine whether an increase in the antibodies and in the globulins parallels one another, or whether either the globulin content or the concentration of immune bodies may increase independently of one another.

Methods.

Healthy Belgian hares kept under constant conditions of diet and activity were used for all the experiments. The animals were fed once daily, the diet consisting of alfalfa hay, toasted bread, oats, and at times small amounts of cabbage. All were allowed a liberal amount of water.

Obtaining Blood.—Specimens of blood, varying in amounts from 5 to 7 cc. were obtained from fresh incisions made in the ear vein. In all instances the use of local applications of xylol or other substances that might cause stasis was avoided. At times the presence of a low blood pressure made bleeding difficult, but in such cases a free flow of blood was obtained by suspending the rabbit by its hind legs. The blood obtained in small sterile tubes was immediately centrifugalized in order to obtain a clear serum. Separation of the serum from the clot was effected as soon as possible, for as will be shown later, serum allowed to remain in contact with the clot is not suitable for accurate determinations of the proteins. The specimens before use were kept in sterile, stoppered vials in the refrigerator.

Tests for Agglutination.—The antigen used for the agglutination tests consisted of a 24 hour carbolized or formalinized Liebig's or rabbit broth cul-

ture, which had been properly controlled by tests with immune sera of known antibody content. To a series of tubes containing the clear, untreated serum in amounts ranging from 0.1 cc. to 0.00005 cc. was added 1 cc. of antigen. After incubating the mixtures for 2 hours at 37° C. or at room temperature for 12 hours readings were made. The final readings were always made after the lapse of 12 or 14 hours. The highest dilution of the serum in which complete agglutination occurred was taken to represent the agglutination titer of that serum, and only these readings are recorded in the tables.

Tests for Complement Fixation. Antigens.—Cultures of the various organisms grown upon lemco broth[1] for 18 to 20 hours[2] were found to be most suitable for this purpose. They were killed by heating for ½ hour at 60°C., and preserved with 0.5 per cent carbolic acid and 1 per cent glycerol. When kept in the refrigerator and protected from the light such antigens may be ready for use after a period of 2 months. Some, however, may become anticomplementary after a period of 4 weeks, and these must be discarded.

The dose of antigen employed was four times the antigenic unit as determined by preliminary titrations of the antigen with a standard amount of immune serum; either 0.1 or 0.2 cc. This dose was at least one-quarter to one-fifth of the anticomplementary unit as determined by repeated titrations. The range of the specific antigenic properties of each antigen was further controlled by tests with sera of known antibody content.

Sera.—Dilutions of inactivated serum (62°C. for 30 minutes) in descending doses from 0.2 to 0.003 cc. were used. In selecting such dilutions it frequently occurred that the gradations were not well chosen. This made it difficult to express the results absolutely in terms of the highest dilution of serum which gave definite fixation. For this reason the signs >< are employed. Thus the notation > 0.005 indicates that complete fixation of the complement would probably have occurred in a serum dilution of 0.004 or 0.003 cc., since in a dilution of 0.002 cc. of the serum only 50 per cent fixation was obtained. In the tables only those dilutions of the serum are recorded which with the proper dose of the antigen caused a complete fixation of the complement.

Hemolytic System.—The anti-sheep hemolytic system was used. Complement was furnished by the pooled sera of several guinea pigs, and was employed in a dosage of 0.05 cc. of a dilution of 1 to 4 in salt solution. The red corpuscles were used in 1 per cent suspension. The hemolytic unit was determined by a preliminary test using 0.05 cc. of complement and 0.5 cc. of a 1 per cent suspension of fresh sheep cells. In the titrations of the antigens as well as for the actual complement fixation tests two hemolytic units (about 0.2 to 0.1 cc. of a dilution of 1 to 100 in saline solution) were employed.

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[1] Eyre, J. W. H., The Elements of Bacteriological Technique; a Laboratory Guide, Philadelphia and London, 2nd edition, 1913, 163.

[2] Cultures less than 18 hours old are frequently inactive, and those older than 24 hours may be anticomplementary in doses of 0.5 cc.

Technique.—Preliminary tests were first made to rule out any anticomplementary activity of the different antigens used. These were carried out as follows: To each of a series of tubes containing decreasing doses of antigen diluted with 1.5 cc. of isotonic salt solution was added 0.05 cc. of complement in a dilution of 1 to 4. The tubes were then incubated at 37° C. either in a water bath for ½ hour or in an incubator for 1 hour. To each tube was then added a previously prepared mixture of two units of hemolysin and 0.5 cc. of the corpuscle suspension. After mixing and incubating for 1 or 2 hours, sedimentation of the red cells was hastened by placing the tubes in the refrigerator so as to make the readings more precise.

After determining the dosage of antigen to be used in the final test, the latter is carried out in the following manner: To each of a series of tubes containing the inactivated serum in descending doses is added 0.05 cc. of complement in 1.5 cc. of saline solution followed by the proper dose of antigen. After incubation for a period of ½ to 1 hour, the sensitized corpuscle suspension is added to each tube in the dosage already given, the mixture reincubated for 1 or 2 hours, and the readings are made as already indicated.

The customary controls for the serum, antigen, and hemolytic system were employed.

Non-specific fixations of the complement by rabbit sera, so frequently observed with bacterial antigens, must, of course, be kept in mind. Such a possibility, however, was ruled out by careful preliminary tests of the serum before immunization with numerous antigens, a procedure recommended by Kolmer and Trist (9). For various reasons it was not possible to select only those rabbits whose sera showed at the outset negative reactions. But we consider that this is unnecessary in serial studies, inasmuch as non-specific fixation does not interfere with specific deviations of the complement due to the presence of immune bodies.

Tests for Antistaphylolysin.—The staphylolysin used in the tests was prepared from a recently isolated strain of *Staphylococcus aureus* grown on a medium having an ionization equal to the value $P_H{}^+ = 7.7$. Two units of this hemotoxin suspended in isotonic salt solution were added to a series of test-tubes containing the inactivated serum in descending doses. The total volume was now made up to 2 cc., and the mixtures were incubated for 15 minutes in a water bath at 37° C. To each tube was then added 0.05 cc. of a suspension of red blood corpuscles prepared by washing the cells and adding an amount of saline solution equal to the original blood volume. The mixtures were now incubated for 1 hour. The readings were made at the end of 2 hours and again after 12 hours, the figures in the tables indicating the dilutions of serum in which the lytic activity of the staphylohemotoxin was completely inhibited.

Quantitation of Serum Proteins.—Those who have heretofore studied the problem of the relationship of the blood proteins to immunity have in the main obtained their data by precipitation of the globulins and the subsequent Kjeldahl determinations of the nitrogen contained in the precipitate and in the coagu-

lated proteins of the whole serum. Other workers have resorted to the less accurate method of weighing the precipitates. Neither of these procedures is applicable to a systematic study requiring frequent observations upon small animals because of the need of large quantities of blood and the time-consuming character of these procedures.

All the determinations of the albumin, globulin, and non-protein constituents in the blood of the animals experimented upon by us were made by the micro-refractometric method of Robertson (10). As the author has shown in numerous publications, the results obtained by this method are in accord with those obtained by the older methods, and the procedure possesses the important advantages of being less laborious and of being applicable to small quantities of serum.

In brief the method is as follows:[3] Blood is collected in centrifuge tubes, allowed to clot, and centrifugalized to obtain a clear serum. The blood should be obtained before a feeding, since lipemic sera are read with more difficulty. Furthermore, the serum should not be allowed to remain in contact with the clot for any length of time, nor should bacterial contamination be permitted, especially if it is desired to keep the serum for 24 or 48 hours before analyzing it.

By actual experiment we became assured that serum, and more particularly immune serum, may dissolve out substances from the clot which may considerably alter its refractive index. It has been found, for instance, that after 48 hours a sterile immune serum[4] kept in contact with the clot at low temperatures already showed a reduction in the protein quotient. On the other hand, the clear serum, immediately separated from the clot and kept under similar conditions, showed no marked changes after a period of 72 hours. A little over 1.5 cc. of serum is sufficient for the determination of the four fractions.

The tests are carried out in glass tubes having an inside diameter of about 5 mm. and walls about 1 mm. thick. These are sealed at one end.

For the determination of the albumin and globulin, 0.5 cc. of a saturated solution of ammonium sulphate is introduced with the aid of a graduated pipette into one of the tubes, about 10 cm. in length. With the same pipette, which has been cleaned by washing with water, alcohol, and ether, and dried by passing through it a stream of cold air, is added the same amount of clear serum. For purposes of mixing, a piece of silver wire is dropped into the tube, a stopper—consisting of a piece of sealed glass tubing inserted into a piece of rubber tubing—is affixed and the mixture of serum and sulphate is now shaken thoroughly. The precipitate of globulin is sedimented by centrifugalization, the clear fluid is diluted with a graduated pipette to one-half and its refractive index determined.

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[3] For details concerning the various steps in the method, and for a discussion of the reasons for them and of the manner of calculating the results, reference should be made to Robertson (10).

[4] This immune serum exhibited a high antibody content. On May 8 the serum agglutinated in a dilution of 1: 4,000, and fixed the complement in a dilution of 0.001 cc. of serum.

This reading corrected for the ammonium sulphate gives the total albumin plus the non-protein.

The non-protein value is determined by mixing in a similar glass tube 0.5 or 1 cc. of the clear serum with an equal volume of 0.04 N acetic acid solution. A short piece of silver wire is now dropped into the tube, the upper end is sealed off in the flame, the mixture shaken, and coagulated by placing the tubes in a beaker of water heated to boiling for several minutes. This precipitate also is sedimented by centrifugalizing and the refractive index of the clear supernatant fluid is determined.

Lastly the refractive index of the whole serum is determined. From this reading the refractive index of the total globulin is obtained by subtracting the refractive reading of the albumin from that of the whole serum after deducting the value of the non-proteins.

The readings were made with a Pulfrich refractometer, and the calculations of the percentages of the various constituents were carried out in the manner presented in detail by Robertson. The results are expressed not only in percentage but also in the per cent of total protein. For purposes of graphic presentation, it was thought well to express the ratio of albumin to globulin in the form of a quotient. This was obtained by dividing the percentage of albumin by that of globulin. Thus a fall in the quotient would indicate a rise in the blood globulins, and *vice versa.*

From a large series of determinations, numbering several hundred, we have become convinced of the accuracy of this method, provided the sources of error are understood and proper care is exercised in the manipulations. The method is especially recommended on account of the rapidity with which the determinations can be made and the small quantities of serum required.

EXPERIMENTAL.

Observations on the serum proteins were made in normal, infected, immunized, and hyperimmunized animals. With the exception of some of the infected animals, a parallel study was made also of the degree of immunity present during different periods of the experiment. As a typical example of an acute infection, staphylococcus pyemia was chosen. Infections with the tubercle bacillus and with sporothrix were selected as types of chronic infections.

For purposes of immunization living and killed cultures of *Bacillus typhosus* and *Bacillus dysenteriæ* (Shiga) and *Staphylococcus pyogenes aureus* were used. In addition to the classical method of immunization, a study was made also of the effect of massive inoculations in normal and immune animals upon the albumin-globulin ratio and

upon antibody formation. To these were added several observations upon the changes produced in the serum proteins by the inoculation of bacterial endotoxins and inflammatory irritants.

Serum Proteins of Normal Rabbits.

Observations on the serum proteins were made on a dozen normal rabbits kept under constant conditions of diet and activity. Notwithstanding the constancy of the conditions, it is apparent that individual animals may show considerable variations in the percentage of the serum proteins. Thus the total proteins may vary from 5 to 7 per cent. The albumin fraction may show fluctuations from 3.1 to 5.5 per cent, and the globulins from 0.8 to 2.7 per cent. But the averages of all the readings yield values which are in fair accord with those of other workers (11), especially in so far as the albumin-globulin ratio is concerned. Such fluctuations as have been observed in the protein quotient in normal animals are small in comparison with the marked diminution in the quotient which has been noted in the pathological conditions studied. Whereas the quotient in normal animals averaged 3.5, and in most instances did not fall below 1.5, infected and immunized animals have at one period or another shown a quotient below 1.0. Furthermore, it is essential in all these experiments to determine the percentages of the serum proteins existing in the normal animal before proceeding to determine the variations which may follow the establishment of a pathological condition.

Infection.

Experiment 1. Infection. Pyemia.—Two rabbits were inoculated intravenously with potato cultures of *Staphylococcus pyogenes aureus*. One of the animals (Rabbit 1) received on February 17 two loopfuls of a culture, and died 8 days later of a bilateral fibrinous pleurisy, and abscesses in the lungs and kidneys. The second animal (Rabbit 2) was inoculated on March 2 with only one loopful of a potato culture. This animal emaciated gradually, losing about 600 gm. in weight, and was killed 13 days later.

Autopsy.—Animal anemic and emaciated. Pleural and peritoneal cavities contain a considerable amount of fluid. Atrophy of mesenteric fat. Slight engorgement of liver and spleen. Abscesses and infarcts in both kidneys. Large abscess in muscles of right hind leg. Fibrinopurulent arthritis of right coxofemoral joint. Bone marrow gelatinous and deep red in color.

The serum proteins were studied at frequent intervals during the course of the infection, and the results obtained showed clearly the fluctuations which may occur in the blood proteins in a typical infection with *Staphylococcus aureus*. The progress of the infection in both animals studied was accompanied by some antibody response as evidenced in the formation of antistaphylolysins. These developed to an equal degree in both rabbits. In Rabbit 2, however, the changes in the percentages of the various constituents, and especially in the albumin-globulin ratio, were not marked. The striking result of this experiment was the precipitous drop in the protein quotient observed in Rabbit 1, which received a massive inoculation of staphylococci. Of interest, too, is the fact that this rise in the total globulins was associated with a parallel fall in the total albumin.

The loss in weight in both animals was considerable, but it is of interest that this loss occurred more rapidly in Rabbit 1 (3 days), whereas as in Rabbit 2 it was more gradual. The significance of this observation will become more apparent in connection with other experiments.

Experiment 2. Tuberculosis.—On February 17 a rabbit weighing 2,600 gm. was inoculated intravenously with 0.1 mg. of a 72 day culture of bovine tubercle bacillus grown on tuberculin agar. During the progress of the infection, frequent analyses of the serum proteins were made, and the albumin-globulin ratio was determined. These observations are presented in Table I.

The animal emaciated only moderately, and was killed about 2½ months later.

Autopsy.—Extensive pulmonary tuberculosis with cavity formation. Numerous cheesy foci and miliary tubercles. Tuberculosis of mediastinal lymph nodes. Tubercles in spleen and kidneys.

Experiment 3. Infection with Sporotrichum schenckii and beurmani.—A rabbit weighing 1,750 gm. was injected intraperitoneally with 2 cc. of a cream suspension of sporothrix (No. 3725, 1912) grown in 4 per cent glucose broth since October 25, 1915. The animal gained in weight and 10 days later 4 cc. of the same culture were injected. On the 30th day a third and final injection of 6 cc. was given. On March 17 three nodules about the size of large cherries were felt at the site of inoculation.

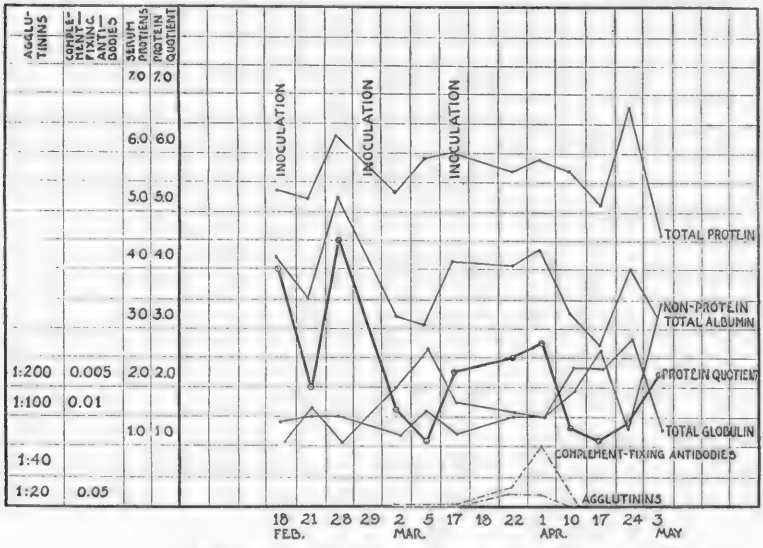
Frequent observations were made upon the agglutinins and complement-fixing antibodies as well as upon the serum proteins (Text-fig. 1). The animal was killed about 2½ months later.

Autopsy.—At the site of the injections there were many pea-sized nodules. These were present both in the subfascial layers and in the abdominal muscles.

TABLE I.

Experiment 2. Chronic Infection. Tuberculosis. Rabbit 3.

Date.	Weight.	Organisms inoculated.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Feb. 17..	2,600	0.1 mg. intravenously.	6.6	5.3	1.3	80	20	1.2	4.0	Bovine strain grown on tuberculin agar, 72 days old.
" 21..	2,400	—	6.0	4.6	1.4	76	24	1.5	3.1	
" 25..	2,625	—	—	—	—	—	—	—	—	
" 28..	2,400	—	6.5	4.7	1.8	72	28	1.6	2.9	
Mar. 9..	2,700	—	6.0	4.2	1.8	70	30	1.4	2.3	
" 17..	2,400	—	6.8	4.6	2.2	68	32	1.4	2.1	
Apr. 1..	2,550	—	7.2	4.4	2.8	61	39	1.5	1.6	
" 10..	2,550	—	6.0	3.1	2.9	51	49	2.6	1.0	
" 17..	2,450	—	5.2	2.1	3.1	40	60	3.5	0.66	
" 24..	2,350	—	6.9	3.6	3.3	52	48	1.7	1.1	
May 3..	2,300	—	5.0	0.5	4.5	10	90	3.5	0.1	Animal killed. Blood for examination obtained from heart.



TEXT-FIG. 1. Infection with sporothrix.

Similar ones were found also in the omentum, between the loops of intestine and between the liver and diaphragm. Histologically, they were found to show the presence of typical sporotrichotic granulation tissue. From one of these nodules a positive culture was obtained.

Both the tubercular and mycotic infections were characterized by a long chronic course of several months associated with only slight wasting. Immediately following the inoculation, in the one instance with the tubercle bacillus and in the other with the sporothrix, each animal showed a slight rise in the total globulins as evidenced by a fall in the protein quotient. But it is of interest that the latter continued at a fairly constant level, and that the fluctuations observed usually followed the intraperitoneal injections. Thus it will be noticed the protein quotient did not show such a precipitous fall in the chronic as in the acute infections.

In the animal infected with sporothrix a slight grade of immunity developed, but neither the agglutinins nor the complement-fixing antibodies ever rose to a high level. Notwithstanding this lack of response, the serum proteins still showed striking fluctuations. The curve of total albumin for the most part paralleled closely the curve of total proteins. The globulins showed a tendency to rise when the albumin curve fell, but this was not the case for all periods of the experiment. Both of these features are well represented by the fluctuations of the protein quotient as shown graphically in the text-figure.

Immunity.

Experiments 1 and 2. Immunization with Bacillus typhosus.—Two healthy rabbits were inoculated with *B. typhosus*. One animal received inoculations of stock strain and the other Army vaccine. Serum samples were obtained before the injection for a study of the agglutinins, complement-fixing antibodies, and serum proteins. Similar observations were made at frequent intervals following the inoculations. The details of Experiment 2, in which Army vaccine was used, are recorded in Table II and Text-fig. 2.

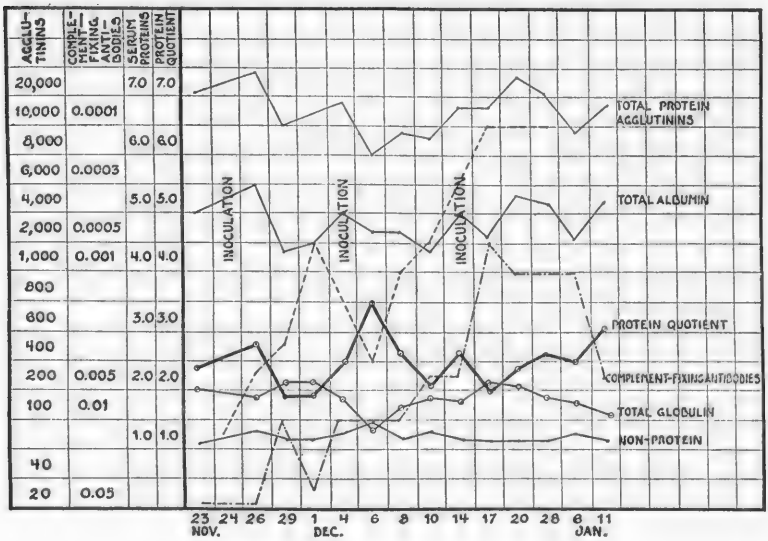
In both of the experiments on typhoid immunization the inoculations were given in such dosage and at such intervals as to develop within the organism a maximum degree of immunity without causing any marked metabolic disorder. Both animals maintained their weight at a normal level throughout the period of immunization, and

TABLE II.

Experiment 2. Typhoid Immunization. Rabbit 4.

Date.	Organisms inoculated.	Antibodies.		Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
		Agglutinins.	Complement-fixing antibodies.								
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Nov. 23.....	—	1:20	0.05	7.0	5.0	2.0	71	29	1.1	2.4	
" 24.....	0.5 cc. (250,000,000) intravenously.	1:80	0.05	—	—	—	—	—	—	—	Army vaccine. Weight 3,800 gm.
" 26.....	—	1:320	0.05	7.4	5.5	1.9	74	26	1.3	2.8	
" 29.....	—	1:500	<0.01	6.5	4.3	2.2	66	34	1.2	1.9	Weight 3,400 gm.
Dec. 1.....	—	1:2,000	0.03	6.7	4.5	2.2	67	33	1.2	2.0	
" 4.....	—	1:800	0.01	6.9	5.0	1.9	72	28	1.3	2.5	
" 5.....	1.0 cc. (500,000,000) intravenously.	—	—	—	—	—	—	—	—	—	
" 6.....	—	1:400	<0.01	6.0	4.7	1.3	78	22	1.4	3.5	
" 8.....	—	1:1,000	<0.01	6.4	4.7	1.7	73	27	1.2	2.7	
" 10.....	—	1:2,000	0.003	6.3	4.4	1.9	69	31	1.3	2.2	
" 14.....	—	1:6,000	0.003	6.8	5.0	1.8	73	27	1.2	2.7	Weight 3,800 gm.
" 15.....	1.0 cc. (500,000,000) intravenously.	—	—	—	—	—	—	—	—	—	
" 17.....	—	1:10,000	<0.0005	6.8	4.6	2.2	67	33	1.2	2.0	
" 20.....	—	1:10,000	0.001	7.4	5.3	2.1	71	29	1.2	2.4	
" 28.....	—	1:10,000	0.001	7 1	5.2	1.9	73	27	1.1	2.7	Weight 3,500 gm.
Jan. 6.....	—	—	0.001	6.4	4.6	1.8	72	28	1.3	2.5	
" 11.....	—	—	0.003	6.8	5.2	1.6	76	24	1.1	3.1	

the immunity developed after the third inoculation was of a high grade in each instance. In one of these animals (Rabbit 5), the agglutinins showed a tendency to fall at one period following an intercurrent infection resulting from an abortion. Soon after this reduction in the agglutination titer, there occurred also a definite rise in the serum globulins. The association of the development of a pyemia with a high globulin content has been a frequent observation. Its significance will be discussed in subsequent paragraphs.



TEXT-FIG. 2. Typhoid immunization.

In neither animal was it possible to demonstrate any direct parallelism between the rise in the immune bodies and the fluctuations in the serum globulins. The latter showed a tendency to rise (fall in protein quotient) 24 to 48 hours following an inoculation, and a tendency to return to a normal level in the following several days.

Text-fig. 2 represents these fluctuations graphically for Experiment 2. It will be observed that after a period of about 3 weeks following the initial inoculation the value of the protein quotient showed no tendency to change materially, although the development of immune

bodies had reached its highest point. The total proteins and total albumins showed parallel fluctuations but no definite tendency to rise during the process of immunization.

Experiment 3. Immunization with Dysentery Bacillus (Shiga).—A rabbit weighing 2,450 gm. was inoculated intravenously with increasing doses of living dysentery bacilli (Strain Do, Pasteur Institute, December, 1913) suspended in salt solution. The first inoculation was given on January 27 when 0.01 of a loop (20,000 organisms) was inoculated. The same dose was given 10 days later. 20 days after the first inoculation the animal received ten times this number of organisms. This was increased to 100 times the dose on the 30th day. On the 40th and 50th days, two and one-third and eight loopfuls, respectively, were inoculated. These injections were all well tolerated and were not followed by loss in weight. The degree of antibody response and the change in the serum proteins are recorded in detail in Table III and Text-fig. 3.

8 weeks after the beginning of the experiment, the animal died of exsanguination following prolonged bleeding from the ear artery.

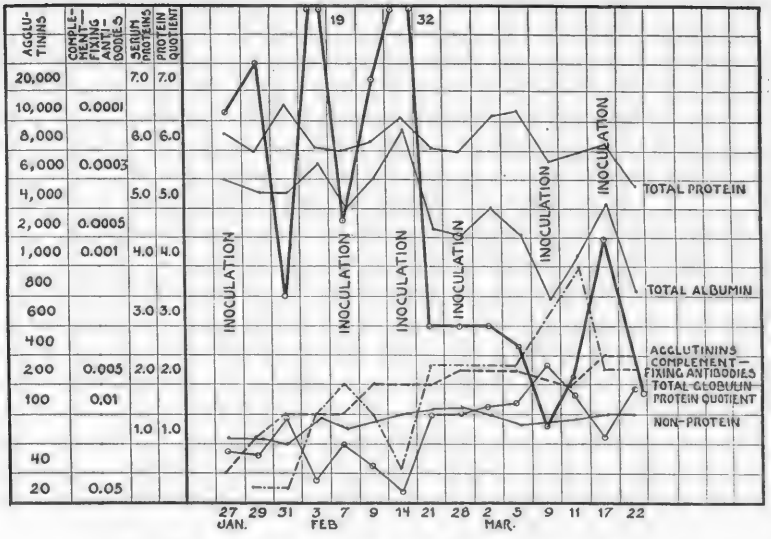
The striking changes in the blood globulins brought about by the inoculation of living dysentery bacilli are well shown in the text-figure. It will be observed that following the first two inoculations both the albumin and globulin curves showed wide fluctuation, and that only after the third inoculation did the globulins show a gradual upward course and the albumin a gradual downward course. During two periods of the experiment (February 3 and 14) the albumin fraction rose to a high level. A similar observation was made upon an animal immunized with living staphylococci. Apart from the explanation that the injection of living organisms may give rise to a marked metabolic disorder, the reasons for such extreme variations in the curve are not clear, unless it is assumed that the active multiplication of bacteria may bear some relation to these fluctuations.

The agglutinins and complement-fixing antibodies rose gradually reaching their highest level during the 5th week. But as a careful analysis of the antibody and globulin curves will disclose, there is a marked fluctuation of the latter curve throughout its entire course. The most striking discrepancy was noted on March 17, when the concentration of antibodies had reached its maximum; whereas the globulin content was beginning to return to its initial level.

TABLE III.

Experiment 3. Immunization with Dysentery Bacillus (Shiga). Rabbit 6.

Date.	Organisms inoculated.	Antibodies.		Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
		Agglutinins.	Complement-fixing antibodies.								
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Jan. 27.....	0.01 loop (20,000) intravenously.	0	0.03	6.3	5.5	0.8	87	13	1.2	6.7	Weight 2,450 gm.
" 29.....	—	1:80	0.03	6.0	5.3	0.7	88	12	1.2	7.5	
" 31.....	—	1:100	0.03	6.8	5.3	1.5	78	22	1.0	3.5	Weight 2,100 gm.
Feb. 3.....	—	1:100	0.01	6.1	5.8	0.3	95	5	1.5	19	
" 7.....	0.01 loop intravenously.	1:100	0.005	6.0	5.0	1.0	83	17	1.3	4.8	Weight 2,300 gm.
" 9.....	—	1:200	0.01	6.2	5.5	0.7	88	12	1.4	7.3	
" 14.....	—	1:200	>0.02	6.6	6.4	0.2	97	3	1.5	32	Weight 2,450 gm.
" 17.....	0.1 loop intravenously.	—	—	—	—	—	—	—	—	—	
" 21.....	—	1:200	<0.003	6.1	4.6	1.5	75	25	1.6	3.0	
" 28.....	—	1:300	<0.003	6.0	4.5	1.5	75	25	1.5	3.0	Weight 2,650 gm.
" 29.....	1 loop intravenously.	—	—	—	—	—	—	—	—	—	
Mar. 2.....	—	—	—	6.6	5.0	1.6	75	25	1.5	3.0	
" 5.....	—	1:300	>0.003	6.7	4.6	1.7	73	27	1.3	2.7	
" 9.....	—	—	—	5.8	3.4	2.4	58	42	1.3	1.4	Weight 2,600 gm.
" 11.....	2.5 loops intravenously.	1:200	0.001	6.0	4.1	1.9	68	32	1.4	2.1	
" 17.....	8 loops intravenously.	1:400	0.003	6.3	5.2	1.1	82	18	1.5	4.5	48 hour culture suspended in 2 cc. of saline solution.
" 22.....	—	1:400	0.003	5.4	3.6	1.8	66	34	1.5	1.9	Weight 2,650 gm.
" 23.....	—	—	—	—	—	—	—	—	—	—	Autopsy. Marked anemia.



TEXT-FIG. 3. Immunization with living dysentery bacilli.

Experiments 4 and 5. Immunization with Living and Killed Staphylococci.— Two healthy animals were inoculated intravenously with *Staphylococcus pyogenes aureus*. One rabbit received 0.001 of a slant (2,000,000 organisms) at the beginning and two subsequent inoculations of 0.02 and 0.1 of a slant. This animal at autopsy showed an osteomyelitis of the sternum, an adhesive pericarditis, and thrombophlebitis of the deep femoral vein. The second animal was injected with cultures of staphylococci killed by heating at 60° C., for one or more hours. In all, four inoculations of 0.01, 0.2, 0.5 and 1 slant were given. The animal died on the 40th day of a septicemia following a hypopyon due to accidental injury.

Some interesting differences have been observed between the effects produced by the intravenous inoculation of living and of killed staphylococci. The injection of living organisms is followed immediately by marked fluctuations in the protein quotient (Text-fig. 3), whereas following the injection of killed organisms the globulins first diminish and then the quotient shows a gradual downward course.

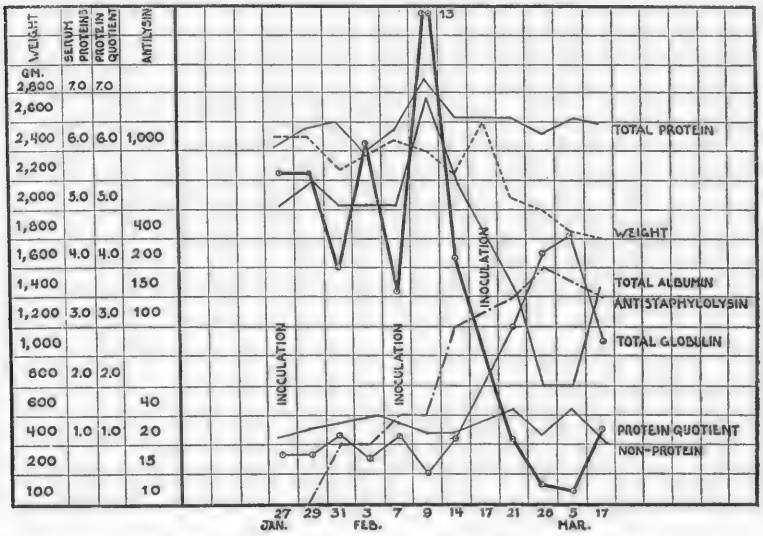
In Experiment 4 the injection of living organisms was followed on the 13th day by a marked rise in the albumin fraction. A similar

TABLE IV.

Experiment 5. Immunization with Killed Staphylococci. Rabbit 7.

Date.	Weight.	Organisms inoculated.	Antibodies.		Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
			Agglutinins.	Complement-fixing antibodies.								
	gm.				*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Jan. 27.....	2,400	0.01 slant intravenously.	0	0	5.8	3.7	2.1	64	36	1.2	1.7	
" 29.....	2,400	—	0	0	5.7	4.2	1.5	73	27	1.5	2.7	
Feb. 3.....	2,350	—	0	0	5.4	4.1	1.3	76	24	1.5	3.1	
" 7.....	2,450	0.2 slant intravenously.	1:10	<0.2	—	—	—	—	—	—	—	Injury on left eye.
" 9.....	2,250	—	1:40	<0.2	6.6	5.7	0.9	86	14	1.4	6.1	
" 14.....	2,300	—	1:100	0.2	6.6	5.9	0.7	90	10	1.4	9.0	
" 21.....	2,600	—	1:60	>0.2	5.9	4.5	1.4	76	24	1.5	3.1	
" 22.....	2,850	0.5 slant intravenously.	—	—	—	—	—	—	—	—	—	
" 28.....	2,550	—	1:100	0.05	6.7	4.2	2.5	62	38	1.6	1.6	
" 29.....	2,600	1 slant intravenously.	—	—	—	—	—	—	—	—	—	
Mar. 2.....	2,600	—	—	—	—	—	—	—	—	—	—	Suspension heated for 1 hr. at 60°C.
" 6.....	2,300	—	1:200	0.01	7.8	2.4	5.4	31	69	1.6	0.45	

event occurred also in Rabbit 7 inoculated with killed organisms. But this animal had developed a suppurative condition of the left eye about the time that this sudden rise occurred. These two observations taken in conjunction with the one following the inoculation of living dysentery bacilli suggest the possibility that such extreme fluctuations may be explained by the active multiplication of living organisms within the animal.



TEXT-FIG. 4. Immunization with living staphylococci.

Both experiments illustrate still another point which will be considered more fully later; namely, that the increase in globulins is associated with a diminution in the albumin fraction. The total proteins exhibit a slight upward course in each animal, whereas the non-protein constituents show no significant variation.

A gradual rise in antistaphylolysins took place in Rabbit 8 inoculated with living organisms. For the most part, this rise appears to parallel the increase in globulins, but we feel that there is another consideration to be kept in mind in the interpretation of this result. The experimental evidence would seem to indicate that marked

alterations in weight, such as occurred in this animal, may be associated with a great increase in the globulins of the blood independently of a rise in immune bodies.

A Comparison of the Effect of the Inoculation of Living Typhoid Bacilli upon the Normal and Immune Animal.

The observations upon the fluctuations in the serum globulins and in the antibody response in normal animals immunized with living organisms suggested the problem of the possible effect of inoculating living organisms into the typhoid immune animal. That the immunized organism because of the sensitization of its fixed tissue cells may possess a more responsive defensive mechanism is now well known (12). This power of defense may become manifest by a rapid mobilization of antibodies and by a large increase in the number of circulating leukocytes. It was our purpose in the experiments of this series to ascertain whether this protective reaction was in any manner related to the changes in the blood proteins, and more especially whether any parallelism existed between the rise in leukocytes and the increase in the blood globulins.

Experiments 1, 2, 3, 4, and 5.—Five animals already possessing a basic immunity against the typhoid bacillus were chosen for this study. In three of the animals (Rabbits 9, 10, and 4) a record was made at intervals of hourly periods of changes in the leukocytes, antibodies, and serum proteins. In one of the experiments (Rabbit 10) the observations were extended over a period of 12 days. The results of this experiment are representative of the others in this series. These are given in detail in Table V and Text-fig. 5.

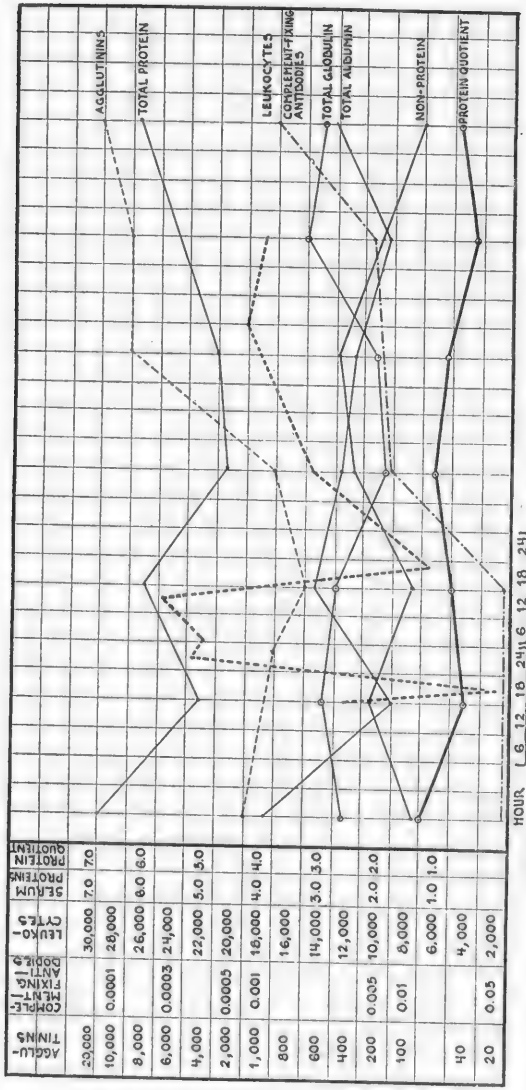
Experiment 6.—A normal rabbit weighing 2,800 gm. was inoculated intravenously with 0.25 of a slant of living typhoid bacilli (Strain H 125) on May 8. The animal tolerated the injection well, and gave a good leukocytic and antibody response. Observations on the leukocytes, antibodies, and serum proteins were made at hourly periods during the first 48 hours, and then at intervals over a period of 16 days.

The observations given in the protocols, tables, and text-figures of this series support the general conclusions that the immune animal responds more quickly to the inoculation of living organisms with a leukocytosis, a rise in immune bodies, and an increase in the blood globulins; and that the changes noted in the blood proteins bear no relation to the hyperleukocytosis.

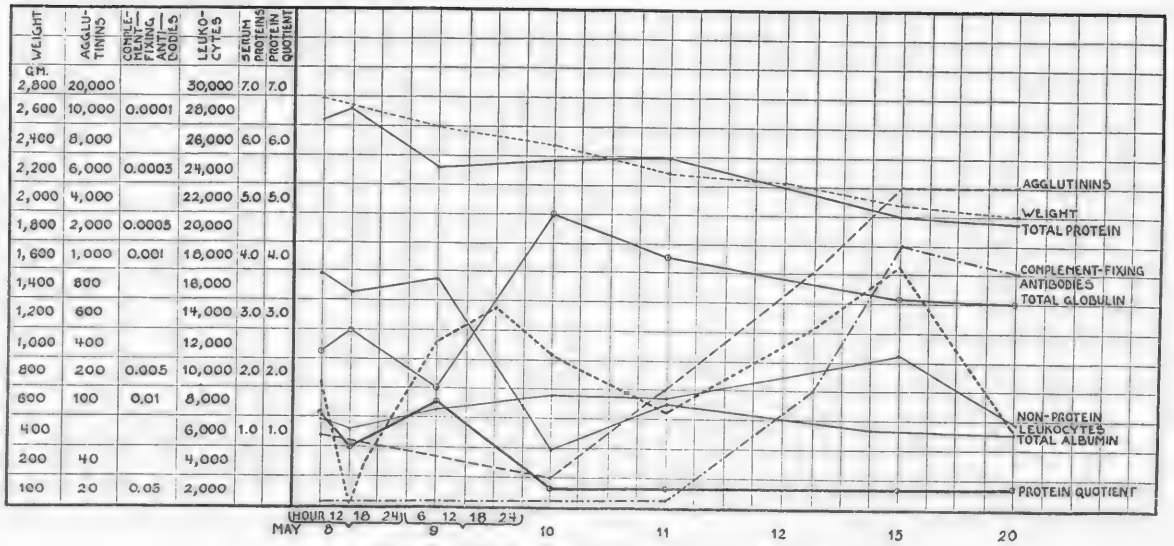
TABLE V.

Experiment 4. Rabbit 10.

Date.	Time.	Weight.	Antibodies.		Leukocyte count.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
			Agglutinins.	Complement-fixing antibodies.									
		gm.				*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Apr. 26.....	—	—	1: 2,000	0.05	—	7.0	4.1	2.9	60	40	1.6	1.5	
" 27.....	12 m.	3,475	—	—	12,300	—	—	—	—	—	—	—	Inoculation of 0.25 slant of 20 hour typhoid culture intravenously.
	4 p.m.	—	—	—	3,770	—	—	—	—	—	—	—	
	5.30 p.m.	—	1: 1,000	0.1++	—	5.3	2.0	3.3	37	63	2.4	0.6	
	8.30 p.m.	—	—	—	23,000	—	—	—	—	—	—	—	. *typhosus* in blood.
" 28.....	9 a.m.	—	—	—	24,600	—	—	—	—	—	—	—	" " " "
	12.10 p.m.	—	1: 800	0.05	18,600	6.2	3.2	3.0	51	49	1.7	1.0	
	6 p.m.	3,350	—	—	7,400	—	—	—	—	—	—	—	
" 29.....	—	—	1: 1,000	0.005++	14,900	4.9	2.8	2.1	57	43	2.6	1.3	
May 1.....	—	3,750	1: 10,000	0.003+++	19,700	5.0	2.7	2.3	54	46	2.7	1.1	
" 3.....	—	—	1: 10,000	0.003+++	19,000	5.7	2.2	3.5	38	62	2.4	0.6	
" 6.....	—	—	1: 20,000	0.001	—	6.3	3.0	3.3	47	53	1.5	0.88	



TEXT-FIG. 5. Injection of living typhoid bacilli. Immune animal.



TEXT-FIG. 6. Injection of living typhoid bacilli. Normal animal.

In the main the details of some of the experiments are given in Table V and Text-figs. 5 and 6, but a few points relating to the individual experiments deserve special mention. From a comparison of the results obtained in the experiments with Rabbits 11 and 12 it would appear that the rapidity of response bears some relation to the degree of the initial basic immunity. The first animal showed a fall in the agglutination titer immediately after the injection, and in this animal the rise in globulins occurred only after 72 hours. In Rabbit 12, however, the antibodies rose steadily, and in this instance a rise in the concentration of the serum globulins took place more rapidly within a period of 24 hours.

Experiments 3 and 4 are more complete since in them the observations were extended over a longer period. That the degree of hyperleukocytosis is dependent in part at least upon the number of organisms inoculated is clear from a comparison of the results obtained in Rabbits 9 and 10. In the former where the more marked reaction occurred, one-half of a standard agar slant had been inoculated; whereas, the latter received only one-quarter of a slant. The highest leukocytic reactions were observed in Rabbit 9 (44,000) and in Rabbit 4 (58,000). In this respect our observations coincide with those of McWilliams (12). The usual leukopenia which immediately follows the intravenous injection occurred in both the normal and immune animal as is graphically shown in Text-figs. 5 and 6.

By referring to these charts it will be noted that in neither animal was there any parallelism between the leukocytic response, the rise in the immune properties of the serum, and the increase in globulins. Whereas the curve showed periods in which a rise in globulins occurred simultaneously with a leukocytosis, the latter remained low even during the periods of leukopenia. Nor was any direct correspondence demonstrable between the rise in immune bodies and the increase in the concentration of the serum globulins. Both in the normal and the immune animal the latter took place long before any appreciable rise in the agglutinins and complement-fixing antibodies had occurred. In Rabbit 10, for instance, a fall in the protein quotient (globulin rise) was demonstrable within 24 hours after the inoculation at a time when the antibody content was at its lowest level; and similarly in Rabbit 13, the normal control, the serum globulins rose mark-

edly within 48 hours, while the antibodies rose to their maximum height only after 4 days. In both instances the globulin content remained high throughout the period of immunization while the antibody curve continued to rise independently of the globulins.

The advantage of such frequent determinations made at different periods during the process of immunization as compared with isolated observations made at random intervals is well illustrated in these experiments. If, for instance, a determination of the globulin fraction should show an increase at a time when the immune bodies had reached a high level, the conclusion would naturally follow that fluctuations in the two parallel one another; whereas, as we have pointed out, more frequent observations demonstrated that such parallelism was not of constant occurrence.

The tabulated results emphasize still another point of importance. To be of absolute value the albumin-globulin ratio must be expressed in terms of their quotient. This takes into consideration also fluctuations which have been found to take place in the total proteins during the course of an infection and during the process of immunization.

The Effect of the Injection of Bacterial Toxin upon the Serum Proteins.

The experimental data already presented afford evidence that both living and killed cultures of various bacteria when inoculated into an animal give rise to marked changes in the serum proteins, and more especially to an upset of the normal albumin-globulin ratio. This phenomenon may well be attributed to the changed condition of the animal's metabolism resulting from the multiplication of bacteria within its body, to the liberation of toxic products from the disintegration of the bacterial bodies, or to both causes. The part which the autolyzed bacterial bodies themselves may play in bringing about the results observed is well shown in the following experiment.

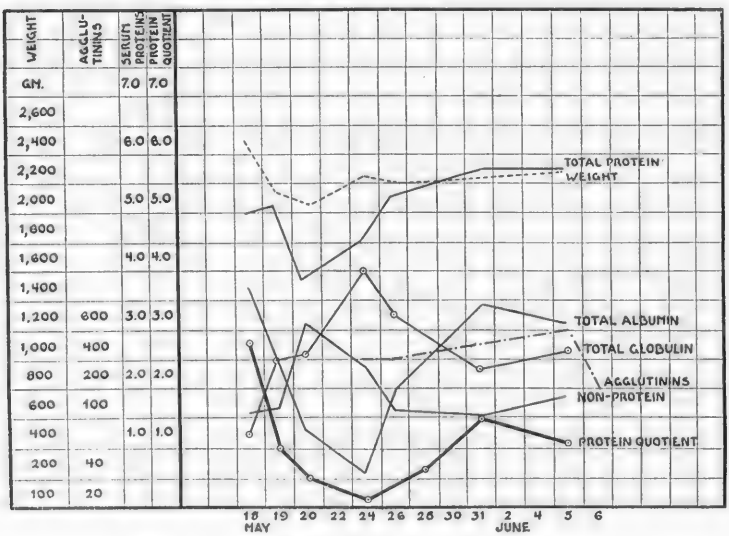
The Action of Bacterial Endotoxin.—A rabbit weighing 2,500 gm. was injected intravenously on May 18 with 1 cc. of the toxin of fowl typhoid. This toxin was prepared by growing the organisms on Martin's broth for 14 days, after which the culture was centrifugalized and filtered through a Berkefeld filter.

Specimens of 5 cc. of blood were taken 24 hours after the injection and at varying intervals until June 6. The alterations in the serum proteins following the injection are given in Table VI and Text-fig. 7.

TABLE VI.

The Effect of the Injection of Bacterial Toxin (Fowl Typhoid). Rabbit 14.

Date.	Weight.	Amount of toxin injected.	Agglutinins.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
May 18...	2,500	1 cc. intravenously.	—	5.0	3.7	1.3	74	26	1.6	2.8	Strain 605 grown on Martin's broth for 14 days. Centrifugalized and filtered once through a Berkefeld filter.
" 19...	2,175	—	—	5.1	2.6	2.5	51	49	1.7	1.0	
" 20...	2,100	—	—	3.9	1.3	2.6	33	67	3.1	0.5	
" 24...	2,275	—	1:400	4.6	0.6	4.0	13	87	2.4	0.15	
" 26...	2,225	—	1:400	5.3	2.0	3.3	37	63	1.7	0.6	
" 31...	—	—	—	5.7	3.4	2.3	60	40	1.6	1.5	
June 5...	2,300	—	1:600	5.7	3.1	2.6	54	46	1.9	1.1	
" 6...	—	—	1:200	—	—	—	—	—	—	—	



TEXT-FIG. 7. The effect of the injection of bacterial toxin.

The dosage of endotoxin in this experiment was apparently well chosen, for although the animal lost moderately in weight, the amount of toxin was not sufficient to prevent a gradual return of the serum proteins to a more or less normal state. The most striking effect of the inoculation, shown graphically in Text-fig. 7, was the gradual increase in the serum globulins at the expense of the albumin fraction, and a reduction in the percentage of the total proteins. This rise in globulins was already appreciable 72 hours following the injection, and 6 days later the albumin-globulin ratio still showed an inversion of the normal formula. On the 8th day the total per cent of proteins had returned to normal, and continued to rise somewhat above the normal level during the subsequent 11 days. At this time the protein quotient, however, still remained low, although the albumin-globulin ratio was beginning to revert to its normal state.

The tendency in this instance for an alteration in the blood protein fractions to readjustment is of interest. In this respect this observation is unique, for in the majority of the experiments an upset of the normal ratio continued with some fluctuations for a long period of time due to subsequent reinoculations. The readjustment of conditions in this animal may be attributed to the absence of bacterial invasion to perpetuate the process. It may be assumed that after its initial effect upon the animal, the toxin was spent, as may be inferred from the appearance of antibodies in the blood, and that the organism was then able to readjust itself.

A Comparison of the Effect of the Intraperitoneal Injection of Bacterial and of Inflammatory Irritants.

In some experiments which will be recorded at a later date, we observed that the intraperitoneal injection of red blood corpuscles gives rise to an alteration in the albumin-globulin ratio which is both rapid in its occurrence and marked in its degree. In fact such injections usually resulted in a complete inversion of the ratio within a period of 24 hours. Because we were dealing here with non-bacterial protein and with a different route of injection, it seemed worth while to ascertain whether it was the nature of the inoculated material or the route of the inoculation which was responsible for

the changes observed. With this purpose in view, the following two experiments were carried out.

Experiment 1.—On May 22 a rabbit weighing 2,525 gm. was injected intraperitoneally with 0.2 of a slant of a killed culture of staphylococci. The organisms were killed by heating for an hour at 60° C., and 1 cc. of the suspension was used. An analysis of the serum proteins was made at stated intervals following the injection. These are recorded in Table VII.

TABLE VII.

The Effect of the Injection of Killed Staphylococci Intraperitoneally. Rabbit 15.

Date.	Weight.	Organisms inoculated.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
May 19..	2,500	—	5.8	3.2	2.6	60	40	1.7	1.5	
" 22..	2,525	0.2 slant intraperitoneally.	—	—	—	—	—	—	—	24 hour culture killed by heating for 1 hr. at 60°C. suspended in 1 cc. of saline solution.
" 23..	2,500	—	4.7	1.3	3.4	28	72	2.9	0.4	
" 24..	2,600	—	4.7	1.8	2.9	38	62	2.8	0.6	
" 26..	2,600	—	4.9	0.8	4.1	16	84	1.8	0.2	
" 31..	—	—	5.7	2.0	3.7	35	65	1.6	0.5	
June 6..	—	—	5.8	1.8	4.0	31	69	1.3	0.45	

Experiment 2.—2 cc. of an aleuronat suspension in saline solution were injected intraperitoneally into a rabbit weighing 2,150 gm. The suspension of aleuronat was so made that it corresponded in density to that of the killed staphylococci used in the first experiment. Following the injection, observations were made upon the serum proteins. These are recorded in Table VIII. The animal lost gradually in weight, and died 7 days after the injection.

Autopsy.—Small masses of unabsorbed aleuronat were found adherent to the peritoneum. There was a definite intestinal paralysis with coprostasis, chiefly in the large bowel. There was considerable injection of the peritoneum and an enteritis of the small bowel.

TABLE VIII.

The Effect of the Injection of Aleuronat Intraperitoneally. Rabbit 16.

Date.	Weight.	Amount of aleuronat injected.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
May 19..	2,075	—	5.1	3.1	2.0	60	40	1.6	1.5	
" 22..	2,150	2cc.	—	—	—	—	—	—	—	Aleuronat (Merck) suspension made in saline solution and of about same density as that of staphylococci (Table VII).
" 23..	2,025	—	4.7	1.8	2.9	38	62	2.8	0.6	
" 24..	2,025	—	4.2	1.7	2.5	40	60	3.1	0.66	
" 26..	1,900	—	4.7	1.5	3.2	32	68	2.6	0.5	
" 29..	1,450	—	—	—	—	—	—	—	—	Death.

Both experiments would seem to support the view that the route of injection rather than the nature of the substance injected is responsible for the rapid inversion in the albumin-globulin ratio. 24 hours following the intraperitoneal injection of killed staphylococci the quotient fell from 1.5 to 0.4, indicating an increase in globulins to more than three times the initial value. This upset in the ratio continued with slight fluctuations for a period of about 2 weeks. The animal injected with aleuronat showed a change similar in every respect.

The retardation in response noted after intravenous inoculations must therefore be attributed to the protective properties of the blood which enable it to delay the action of the bacteria or toxin upon the body tissues.

Another point brought out by this experiment deserves emphasis; namely, that agents other than bacteria or their toxins may cause an upset in the serum proteins. The manner in which an inflammatory irritant and leukotactic substance like aleuronat may produce this result offers some difficulty of explanation. It is not unlikely, however, that the rapid absorption of toxic protein products result-

ing from the disintegration of leukocytes and fixed tissue cells produces a profound metabolic disturbance of which the heaping up of blood globulins is one of the resultant phenomena. A further consideration of the factors which may give rise to this result will be presented later.

DISCUSSION.

The experimental evidence presented does not support the views held by a number of workers concerning the relationship of the blood globulins to the resistance developed in bacterial infection and immunity. From a large number of observations, continued over a long period of time, we have become convinced that other causes are responsible for the rise in globulins observed in these conditions.

Our observations have shown with considerable certainty that a heaping up of globulins in the blood during the development of an infection is more apt to occur in those instances where the infection has been overwhelming and associated with extensive suppuration and wasting. We have found, in fact, that animals which succumb to such an acute process have usually developed only a moderate resistance as far as the development of immune bodies is concerned. On the other hand, a mild chronic infection may continue over a long period of time, and may register only slight changes in the blood globulins until the animal begins to emaciate and to lose in weight. This point has been discussed in connection with the tubercular and the mycotic infections.

The main points of interest have come from a study of the serum globulins during the process of immunization. Contrary to the results of a number of workers (13), our experiments have shown that immunization with bacteria causes a rise in globulins only when the animals react severely to the inoculation. Immunization carried out carefully and with a well controlled dosage is not usually accompanied by an increase in the serum globulins, although the immune bodies may attain a high concentration. The inoculation of massive doses, however, either into a normal animal or into an animal already possessing a basic immunity results in most instances in a marked rise in the globulins. This may occur, indeed, before the animal has responded by the production of antibodies. It would seem,

therefore, that no direct parallelism exists between the two phenomena. In fact we have come to regard the heaping up of serum globulins supervening during the process of immunization as an index of a metabolic disorder unfavorable to the attainment of the best immunologic results. And it is not unlikely that observations on the blood globulins may serve as an important practical guide to careful immunization.

Any attempt to explain the cause of the rise in globulins observed in infection and immunity is difficult. At best all such explanations must be of a hypothetical character until we have learned more concerning the origin of the various protein fractions, their function,[5] and their chemical nature. One important conclusion may be derived from the experimental evidence presented; namely, that the increase in blood globulins is usually accompanied by a marked metabolic disorder. This observation has been made also by other workers (14). Clinically, the metabolic disturbance is manifested by a febrile reaction, intoxication, and rapid emaciation. The extensive destruction of body protein which is going on is further evidenced by an increase in the nitrogen elimination (15). That such marked proteolytic activity may be initiated by the intravenous injection of bacteria, bacterial toxins, and protein split-products has been clearly shown by Jobling and his coworkers (16). They attribute this active proteolysis to a more or less marked mobilization of ferments, both protease and lipase. Apart from the consideration of the cause of this disturbance in metabolism, it seems reasonable to assume that it must register a change in the proteins of the blood.

More difficult to explain, however, are the facts that an inversion of the albumin-globulin ratio can be so readily produced, and that the change markedly affects the globulins. The possible explanations which may be offered for these phenomena have some basis in experiment. Moll (17) has shown, for instance, that under optimum conditions of reaction and temperature crystalline albumin can be converted *in vitro* into a substance whose chemical and physical

[5] Friedemann (*Z. Hyg. u. Infectionskrankh.*, 1910, lxvii, 279) thinks that the globulins and albumins of normal serum are in antagonism, the albumins preventing certain reactions, such as complement fixation, in which the former become active as soon as the albumins are removed or diminished.

properties correspond in every particular to a globulin. On the basis of this observation one would have to assume that the more rapid conversion of albumin into globulin within the body is only a part of the accelerated metabolism which takes place in infected and immunized animals.

This is only one of many hypotheses which might be advanced to explain the difficult questions which the recorded observations offer for consideration. But at the present time it is better to adhere to those views which have an experimental basis until further additions to our knowledge make them untenable.

SUMMARY.

The progress of an infection is usually associated with marked changes in the serum proteins. There may be an increase in the percentage of the total protein during some stage of the infection, and there is usually a change in the albumin-globulin ratio with an increase in the total globulins. This rise may antedate the development of any resistance by a considerable period of time.

The non-protein constituents of the blood show fluctuations with a tendency to rise as the infection progresses.

The process of immunization is in almost all instances associated with a definite increase in the globulins of the blood, and in some cases with a complete inversion of the normal albumin-globulin ratio. This may be produced both by living and dead organisms and by bacterial endotoxins. Massive doses usually result in an upset which shows no tendency to right itself during the period of observation.

A rise in the globulins has been shown to occur long before the animal develops immune bodies in any appreciable concentration; and where the globulin curve and antibody curve appear to parallel one another, it can be shown by a careful analysis of both curves that there is a definite lack of correspondence at various periods of the experiment.

Animals possessing a basic immunity show a more rapid rise in the globulin curve following inoculation.

There is no parallelism between the leukocytic reaction and the

globulin reaction. During periods of leukopenia the globulins may be as high as during the period of a leukocytosis.

Bacterial endotoxins produce as striking an increase in the serum globulins as do living and killed bacteria. This would seem to indicate that a bacterial invasion of the organism is not absolutely essential for the globulin changes, and that the toxogenic factor in infection and immunity must play a part in the production of the changes noted.

Inflammatory irritants injected intraperitoneally also result in a globulin increase. In this case the changes produced may best be explained by the toxogenic effect produced by the protein split products resulting from the inflammatory condition.

Intraperitoneal injections of killed bacteria give rise to a more rapid increase in the serum globulins. The rapidity of the response following intraperitoneal as compared with intravenous injections doubtless stands in intimate relationship to the neutralizing power possessed by the blood serum and perhaps to the more extensive surface of absorption following injection by the intraperitoneal route.

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THE RELATION BETWEEN THE THYROID AND PARATHYROID GLANDS.

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PLATE 42.

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Although the thyroid and parathyroid glands are generally regarded as independent organs, numerous observations seem to indicate a functional cooperation between them. These views are based essentially on the microscopic changes that take place in one of the glands after entire or partial extirpation of the other. Although both glands under normal conditions present different and characteristic structures, they may, especially the thyroid gland, under various experimental conditions, undergo structural changes.

The opinions generally held regarding this question are as follows: (1) After parathyroidectomy hypertrophy of the thyroid gland develops (Edmunds,[1] Vassale and Generali,[2] Halpenny and Thompson[3]). (2) After complete removal of the thyroid gland with the enclosed parathyroidea interna, hypertrophy of the parathyroidea externa occurs, with a tendency to the formation of colloid alveoli; in other words, the structure becomes like that of the thyroid (Halpenny and Thompson,[3] Vincent and Jolly,[4] Blum,[5] and Kishi[6]). For these rea-

[1] Edmunds, W., Further Observations on the Thyroid Gland (VI.) *J. Path. and Bacteriol.*, 1907–08, xii, 101.

[2] Vassale, G., and Generali, F., Fonction parathyréoïdienne et fonction thyréoïdienne (Résumé), *Arch. ital. biol.*, 1900, xxxiii, 154.

[3] Halpenny, J., and Thompson, F. D., On the Relationship between the Thyroid and Parathyroids, *Anat. Anz.*, 1909, xxxiv, 376.

[4] Vincent, S., and Jolly, W. A., Some Observations upon the Functions of the Thyroid and Parathyroid Glands, *J. Physiol.*, 1905, xxxii, 65; Further Observations upon the Functions of the Thyroid and Parathyroid Glands, *ibid.*, 1906, xxxiv, 295.

[5] Blum, F., Die Schilddrüse als entgiftendes Organ, *Virchows Arch. path. Anat.*, 1899, clviii, 495.

[6] Kishi, K., Beiträge zur Physiologie der Schilddrüse, *Virchows Arch. path. Anat.*, 1904, clxxvi, 260.

sons most investigators regard both glands as cooperating physiologically, but not as identical.

Before reporting my experiments, I shall describe the changes that may occur in the thyroid that has not been subjected to surgical procedure. We know from earlier observations that the thyroid gland reacts macroscopically and microscopically to various influences. That it sometimes increases in size during pregnancy has long been known. Bircher[7] has produced hypertrophy experimentally in rats and dogs by giving them water from districts where goiter is prevalent among the inhabitants. Halsted[8] was among the first to call attention to the frequent appearance of hypertrophy of the thyroid gland in dogs, without being able to give a satisfactory explanation in all cases.

In a previous publication[9] I showed that an excessive meat diet will cause constant changes in the thyroid glands of rats. The changes consist chiefly of an increase in the weight of the glands (50 per cent), of a proliferation of the epithelial cells, and of a decrease of the colloid substance. Similar experiments with the same results have been previously performed by Watson[10] and others.

The Thyroid Gland after Meat Diet.

Thyroid glands were taken from five cats which for a period of from 8 to 15 months had lived in the laboratory entirely on a diet of meat and water. The same changes previously observed in rats occurred in the cats; *i.e.*, enlargement of the gland, proliferation of the cells, and disappearance of the colloid substance (Fig. 1).

The enlarged gland was weighed in only one instance. This animal, which weighed 3,100 gm., died during an operation as a result of the

[7] Bircher, E., Zur experimentellen Erzeugung der Struma, zugleich ein Beitrag zu deren Histogenese, *Deutsch. Z. Chir.*, 1910, ciii, 276.

[8] Halsted, W. S., An Experimental Study of the Thyroid Gland of Dogs, with Especial Consideration of Hypertrophy of This Gland, *Johns Hopkins Hosp. Rep.*, 1896, i.

[9] Tanberg, A., Om virkrungen ad ensidig kjödernoring, sorlig par glandula thyroidea, *Norsk Mag. Lægevidensk.*, 1910, viii, 516.

[10] Watson. D. C., The Rôle of an Excessive Meat Diet in the Induction of Gout, *Lancet*, 1905, i, 347.

anesthetic, after living on meat for 15 months. In this case the thyroid gland weighed 0.97 gm., and was thus more than three times as large as normally (0.25 to 0.30 gm.). In the other animals the enlargement can only be roughly estimated, but it became evident by a later operation that the thyroid gland was enlarged to about double the normal size.

The proliferation of the cells was pronounced in all cases, although the details varied microscopically. Instead of the regular alveoli lined with a single layer of cells, irregular and often ramified spaces were found. These are produced by proliferation of the epithelium which retains the character of a single layer only in a few places, and protrudes from the wall in solid broad masses, as bud-like proliferations, like partition walls of irregular and sometimes ramified forms. The more compact formations often give rise to small round cavities that probably represent the formation of new alveoli. In these cases the alveolar structure is thus fairly preserved, but in other cases one finds a proliferation which is so strong that the tissue is quite solid, and only an irregular space indicates the remains of earlier alveolar cavities. In the parts where the tissue is more compact the cells lie closely packed without any characteristic arrangement, but in some places there seems to be a tendency toward the formation of small alveoli. The increase of the interstitial connective tissue is negligible. The colloid substance had disappeared and the alveoli are filled with a transparent or slightly granular substance.

It will appear from this description that the hypertrophy caused by a meat diet corresponds with the hypertrophy that develops in the small pieces of the thyroid gland which are left after incomplete thyroidectomy. This is shown in Fig. 2, from a cat on which 10 months previously a bilateral thyroidectomy was performed with retention of both parathyroid glands. After the operation the animal was doing well, and there were no symptoms of tetany or cachexia. At autopsy small pieces of thyroid tissue the size of a pea were found.

On microscopic examination the epithelial layers are seen to have multiplied in many places and to protrude in the form of partition walls and irregular proliferations. No colloid substance was found in the alveoli.

The similarity of the two microscopic pictures is evident. The pro-

liferation of the cells seems to take place according to the same principle, and in both cases the colloid substance also disappears. It therefore seems justifiable to conclude that an excessive meat diet gives rise to an increased activity of the thyroid gland.

The Parathyroid Gland after Meat Diet.

In the animals which had been fed on meat for a long time, the parathyroid gland was also examined in order to find possible changes in the macro- and microscopic appearance. On the whole, microscopically the parathyroid gland is normal in appearance, especially as regards the arrangement of the cells. Small syncytial cells are rare, but these may also be absent in normal glands. Hence relatively large cell forms are generally observed. It should be emphasized that the cells of the parathyroid gland do not react in the same way as those of the thyroid gland on an excessive meat diet, and we may therefore conclude that there is a difference in the functions of the cells of these organs.

Whether an increase in the size of the parathyroid gland occurs cannot be determined by the experiments performed. In organs of such small dimensions and so irregular in appearance, it is difficult to ascertain whether the size is normal.

It is possible that the size of the parathyroid gland may vary according to the diet and way of living. In this connection it should be recalled that a considerable difference is found in the weight of the parathyroid gland in man and in oxen. Vincent[11] gives the average weight of each of the glands in man as 0.035 gm. I have removed the glands in oxen for opotherapeutic experiments and noted that they were considerably smaller than in man. The average weight was 0.021 gm. It is not improbable that differences in diet and metabolism may account for the relatively small parathyroid in oxen.

I have attempted to show that an excessive meat diet may develop hypertrophy of the thyroid gland. This does not mean that other causes for similar changes in the gland may not exist, and the results of surgical operations must be confirmed microscopically by serial sec-

[11] Vincent, S., Innere Sekretion und Drüsen ohne Ausführungsgang, *Ergebn. Physiol.*, 1911, xi, 218.

tions in all cases. This is especially necessary where the larger part of the organs has been extirpated, and where it is of importance to identify all the small remaining pieces.

I shall not mention here the results of experimental extirpation of the parathyroid and thyroid glands from the clinical point of view. I shall merely state that complete parathyroidectomy always resulted in fatal tetany, and complete thyroidectomy in chronic cachexia. Fatal tetany can be prevented only by leaving part of the parathyroid gland, and part of the thyroid must be left in order to prevent cachexia thyreopriva. I shall report only the experiments which seem to indicate a mutual relation between the glands, as far as this can be determined microscopically. The investigations will therefore deal with the changes that occur in one of the organs after entire or partial extirpation of the other.

The Appearance of the Thyroid Gland after Parathyroidectomy.

When the experimental animals died of acute tetany in the course of the first few days after parathyroidectomy, no noteworthy changes were found in the structure of the thyroid gland. The gland had in all cases the same appearance as at the time of operation. The result is different when a protracted insufficiency occurs after the operation, especially in chronic tetany.[12]

In three of my experimental animals chronic tetany developed after extirpation of three parathyroid glands. The animals died after 8, 10, and 11 months, respectively, and there was thus ample opportunity of observing the results that insufficiency of the parathyroid gland would cause in the macro- and microscopic appearance of the thyroid gland. The same results were obtained in all three cases.

The thyroid gland was not enlarged; it was possibly a little smaller than normally. Microscopic examination shows that the alveoli which are normally filled with colloid substance are considerably smaller, and of a round, regular shape, and the cells are generally small and low. Compact bits of intervesicular tissue without cavities are also seen, which probably arise from the relatively small

[12] Tanberg, A., Ueber die chronische Tetanie nach Extirpation von Glandulae parathyreodideae, *Mitt. Grenzgeb. Med. u. Chir.*, 1914, xxvii, 575.

dimensions of the alveoli, whereby the section is apt to traverse compact parts of the gland.

According to this the alveoli in the thyroid gland in chronic tetany should be smaller than usual. This, in connection with the relatively small dimensions of the cells, seems to point to a lower activity of the gland by chronic insufficiency of the parathyroid gland; or, in other words, in chronic tetany the thyroid gland becomes slightly atrophied.

The following questions naturally arise in this connection: Is it not possible that this atrophy, which in all cases is slight, is caused by the chronic disease which has lasted during several months, and is it not possible that any other disease of long duration might give rise to the same changes? Is this atrophy specific? Is it due solely to the extirpation of the thyroid gland?

Before taking up these points, I shall mention a peculiarity in the microscopic picture. From the experiments described above it is seen that hypertrophy of the thyroid gland occurs after a protracted meat diet, and no hypertrophy exists in animals suffering from chronic tetany (Fig. 3). On the contrary, atrophy is evident, and yet these animals have for a period of 8 to 11 months lived on meat, which under normal conditions would be sufficient to develop the changes characteristic of this diet. Hence it appears that a meat diet does not cause hypertrophy of the thyroid gland when an insufficiency of the parathyroid gland exists at the same time.

We shall return to the first question. Is the specific cause of the phenomenon to be found in the extirpation of the parathyroid gland? The following observations from Cat 31 support the view that extirpation of the parathyroid is able to influence the structure of the thyroid gland.

Four parathyroid glands were removed on Sept. 22, 1910. After the operation a slight transient tetany ensued. The weight had increased during the last 5 months, and when the animal was killed on Oct. 28, 1911, there were no signs of disease. The cat had lived entirely on meat for 13 months.

On microscopic examination a fifth parathyroid was found enclosed in the thyroid. The thyroid was of normal size (weight 0.28 gm.). Microscopically no proliferation of the cells was found. The structure was, on the whole, of a normal appearance with regular alveoli of ordinary size filled with colloid substance.

In this case the extirpation of four parathyroid glands did not cause chronic tetany, because the fifth remaining gland was sufficient to prevent its development. But in spite of a protracted meat diet no hypertrophy of the thyroid gland occurred, and no other cause could be detected than the extirpation of the parathyroid gland which had been performed 13 months previously. It therefore may be concluded that even if extirpation of the parathyroid gland is not followed by clinically evident signs of insufficiency, a meat diet does not under these circumstances develop hypertrophy of the thyroid gland.

Cat 32 furnished even stronger evidence of this fact. This animal which had been fed on meat for a long time, was operated on Sept. 26, 1910. Two parathyroid glands and some small pieces of the thyroid were removed, which showed microscopically the characteristic signs of a meat diet. On Feb. 13, 1911, the entire right thyroid gland was removed. It presented the typical appearance of hypertrophy caused by a meat diet; *i.e.*, disappearance of the colloid substance, and a pronounced proliferation of the cells, which transformed the entire gland into a compact tissue. Hence extirpation of two parathyroids could not influence the appearance of the thyroid gland. The last operation was followed by a protracted tetany, which, however, was finally cured.

On Oct. 4, 1911, the middle third of the left thyroid gland was removed. Microscopic examination showed that the thyroid gland had lost its previous appearance. The hypertrophy caused by a meat diet had diminished, although the animal had still been fed exclusively on meat. Instead of the earlier compact proliferation small regular alveoli now appeared, being perhaps a little smaller than normally and lined with a single layer of small cuboidal cells. Colloid substance had again collected in the alveoli.

In this case microscopic pictures are seen of three different stages. At the beginning of the experiment the hypertrophy characteristic of a meat diet was present, and the extirpation of two parathyroid glands did not cause any change. After the third parathyroid gland had been removed together with one-half of the thyroid gland, symptoms of insufficiency occurred, which were finally cured. When the animal was operated on for the third time, nothing abnormal could be observed, and it became apparent that the hypertrophy had diminished and that the thyroid gland was almost normal in appearance.

The cause for this change must be found in the operation performed on Feb. 13, 1911, at which the right thyroid gland and the third parathyroid enclosed in it were removed. Extirpation of one of the thyroid glands cannot have caused the changes in appearance, as this, on the contrary, should produce hypertrophy of the remaining gland. The cause must undoubtedly be looked for in the last operation which resulted in a lasting insufficiency of the parathyroid gland. Although this had apparently been removed, the thyroid gland had nevertheless changed its structure. The conclusion may therefore be drawn that in a thyroid gland where pronounced hypertrophy is already present because of a meat diet, this disappears and the gland assumes its normal appearance after the extirpation of a sufficiently large number of parathyroid glands (in this case three).

From these examples it will be seen that extirpation of the parathyroid gland influences the microscopic appearance of the thyroid gland, and that it seems to be of comparatively little consequence whether hypertrophy caused by a meat diet is present or not. It seems to indicate that an insufficiency in the parathyroid gland corresponded to a certain picture of the thyroid, and as this picture is probably the result of a certain degree of functional activity, we come to the conclusion that an insufficiency in the parathyroid gland develops functional changes in the thyroid gland.

This insufficiency need not necessarily be pronounced enough to give clinically distinct symptoms. The thyroid gland, which easily reacts to influences leading to changes in its function (diet, pregnancy, and water from districts where goiter is prevalent among the inhabitants), is also highly sensitive to a reduction in the function of the parathyroid gland. The small alveoli with cuboidal cells and the absence of hypertrophy caused by meat diet seem to imply some loss of function, or, in other words, that an insufficiency of the parathyroid gland inhibits the functions of the thyroid gland. The view that the functions of the parathyroid and thyroid glands are specific does not, however, exclude the occurrence of a functional interaction between the two glandular systems. But it would be difficult to determine its nature and scope on the basis of these experiments alone.

Gley,[13] and more recently, Vincent, Jolly, and others have already suggested a functional cooperation which they believe to be of a vicarious nature. In our experiments, on the contrary, the results of chronic tetany, as well as the influence of parathyroidectomy on hypertrophy caused by a meat diet, seem to imply that insufficiency of the parathyroid gland causes a reduction of the functions of the thyroid gland.

The Appearance of the Parathyroid Gland after Thyroidectomy.

The material for examination was taken from three cats on which complete thyroidectomy was performed with maintenance of both external parathyroids. All the animals had cachexia thyreopriva, with apathy, fall of temperature, etc. They lived from several months up to 1 or 2 years after the operation.

On microscopic examination serial sections from two cases showed no trace of remains of the thyroid gland. In the third animal a small piece was found, hardly larger than a parathyroid gland, which probably had been instrumental in keeping the animal alive during the 3 years when it was under observation, but which had not been sufficient to prevent the development of definite symptoms of thyreopriva. The animal never showed any signs of tetany, in spite of the insufficiency of the thyroid gland, the reason evidently being that both external parathyroids were left.

In all three cases both external parathyroids were identified in serial sections. There was no essential change in their appearance. Microscopically no structure was found resembling the thyroid gland. No increase in the scant colloid substance nor any formation of follicles has been found. The appearance was normal in spite of the fact that the animals lived for a long time with a constant insufficiency of the thyroid gland, and the parathyroid, therefore, should have had ample opportunity to modify its structure and function, if it had this power.

On the other hand, the glands, as a rule, seemed to be larger than usual. In all probability a compensatory hypertrophy developed as a

[13] Gley, E., Recherches sur la fonction de la glande thyroïde, *Arch. physiol. norm. et path.*, 1892, iv, 311.

result of the extirpation of the internal glands. The actual measurements of the hypertrophy cannot be given.

No definite type of cells can be mentioned as characteristic of the hypertrophied gland. As a rule, however, the cells and the nuclei are relatively large and more symmetrical, while in the normal glands the cells are, as a rule, of various sizes, from syncytial-like cells with little protoplasm to the large transparent cells. The cells in the hypertrophied glands are always of the larger variety, and forms with transparent contents as well as forms with more granular protoplasm are to be seen. Oxyphil cells are not observed here or in the normal glands.

These experiments seem to indicate that the parathyroid gland does not undergo structural changes after total thyroidectomy.

Hypertrophy of the Parathyroid Gland.

The comparatively small changes occurring in the external glands after removal of both the internal glands and both thyroids have already been described. It would be natural to assume that these changes would be more pronounced if the animal had only one remaining parathyroid gland and had lived under a constant insufficiency, as is the case in chronic tetany.

It is also evident that the microscopic picture of the parathyroid gland as it appears in this disease does not in all cases agree with the one found in simple hypertrophy where the extirpation has not been extensive enough to give rise to a chronic insufficiency.

Cats suffering from chronic tetany show on microscopic examination that the remaining parathyroid gland may sometimes assume an appearance which does not present any point of resemblance to the previous descriptions of hypertrophied glands (Fig. 4).

Even under low magnification the microscopic aspect is pronounced in large transparent cells. Under normal conditions the parathyroid cells (Fig. 6) are comparatively little larger than those of the thyroid gland. This is also the case with the nuclei; often hardly any difference in this respect is observed. In this case, however, the contrast in the dimensions of the cells is striking, and is furthermore emphasized by the fact that the thyroid cells in chronic tetany are, if anything, smaller than usual.

With high magnification the following details can be seen: The cells are large, of round, angular, or irregular shape, and with distinct cellular limits. The nuclei are large, eccentric, and rich in chromatin; as a rule, they are round or oblong, but angular forms are frequently seen. The contents of the cells are somewhat varied, but the most characteristic feature is that the greater part of the contents, which surrounds the nuclei to a greater or less extent and often fills the entire cell, is colorless and transparent. Generally along the walls of the cells a small border of granular or filiform protoplasm is seen which without any distinct limit runs into the central transparent part. Sometimes the granular protoplasm is more distended, but even then a more transparent zone can be seen around the nucleus. Distinct vacuoles are nowhere to be seen. The vascularization is ample, and the perivascular tissue somewhat increased, so that the substance of the gland assumes the appearance of being divided into several otherwise not very distinct lobules. No round cellular infiltration is seen.

As already mentioned, the cells, as well as the nuclei, are considerably larger than those generally observed under normal conditions (Fig. 6). While the normal cells of the parathyroid glands measure from 6 to 12μ in diameter, the cells in this instance ranged from 12 by 15μ to 21 by 24μ, or about double the normal diameter. That the nuclei also considerably exceed the normal size is shown in Fig. 4.

Hypertrophy of the Remaining Parts of the Thyroid Gland.

It is well known that when large parts of the thyroid gland are removed, the remaining parts become hypertrophied. Fig. 2 shows distinctly the more characteristic features of this hypertrophy. This form of hypertrophy generally occurs in cases where the remaining parts of the gland have been so large that they are sufficient to prevent the development of cachexia thyreopriva. However, when the remaining pieces are too small to prevent these symptoms, the proliferation of the cells is more pronounced.

Fig. 5 shows the small remaining parts of thyroid tissue found in animals which have suffered for a long time from cachexia thyreopriva.

The gland is for the most part transformed into compact tissue, con-

sisting of closely packed, sharply defined cells of irregular, round, oval, or angular shapes, with nuclei of various sizes rich in chromatin. The protoplasm is also often heterogeneous, in that the cells with strongly granular protoplasm alternate with cells of more transparent and more homogeneous contents.

The arrangement of the cells is usually quite irregular. In several places it is evident, however, that they have retained a tendency to form alveoli, as one often finds small cavities which are more or less filled with granular or filiform contents or sometimes apparently empty. Surrounding these cavities, a long row of evenly arranged cuboidal cells may be observed. Colloid substance is only exceptionally found in the cavities. The tissue is pierced by an ample network of thin anastomosing strings of connective tissue, which in certain places, in the form of thick bundles, divide the gland into distinct lobules. This picture undoubtedly presents certain points of resemblance to the parathyroid gland. It differs in the more irregular shape and arrangement of the cells, in the richer development of connective tissue, and in the pronounced tendency to form small alveoli. Unless serial sections of the remaining parts of the thyroid are examined they may be mistaken for a parathyroid, which structurally shows a transition picture to the thyroid gland.

CONCLUSIONS.

The following conclusions may be drawn from the experiments presented in this article.

1. Excessive meat diet develops hypertrophy of the thyroid gland. A definite hypertrophy of the parathyroid gland under the same conditions has not been established. A meat diet does not develop hypertrophy of the thyroid gland when insufficiency of the parathyroid gland exists at the same time, even if no clinical symptoms are present. Where a pronounced hypertrophy caused by a meat diet has already developed, the hypertrophy disappears and the gland assumes its ordinary appearance after extirpation of a sufficiently large number of parathyroid glands.

2. After parathyroidectomy no hypertrophy of the thyroid gland takes place. In chronic tetany the thyroid gland seems, on the contrary, to atrophy in spite of a meat diet.

3. After complete extirpation of the thyroid gland, the parathyroid gland does not change its structure, even in cases where the cachexia lasts for several years. Small remaining parts of the thyroid gland may through hypertrophy develop into compact tissue and thereby seemingly present some points of resemblance to the parathyroid gland.

4. When the parathyroid gland hypertrophies, as in some forms of chronic tetany, this hypertrophy is characterized by the development of large, transparent, sharply defined cells, with large nuclei rich in chromatin.

5. The parathyroid and thyroid glands are independent organs, each having specific functions. This, however, does not exclude the occurrence of a direct or indirect interaction in the functions of the two systems.

6. There is reason to believe that an insufficiency of the parathyroid gland checks to some extent the function of the thyroid gland. No proof of the existence of a vicarious cooperation between the two glands has been established.

EXPLANATION OF PLATE 42.

FIG. 1. Hypertrophied thyroid gland in a cat after 15 months on a meat diet. × 150.

FIG. 2. The remaining parts of the hypertrophied thyroid gland in a cat in which cachexia did not develop after partial thyroidectomy. × 150.

FIG. 3. Atrophied thyroid gland in a cat with chronic tetany. The colloid substance present in the alveoli cannot be seen in the photograph. × 150.

FIG. 4. The parathyroid gland in a cat with chronic tetany. × 150.

FIG. 5. The remaining parts of the hypertrophied thyroid gland in a cat with chronic cachexia. × 150.

FIG. 6. Normal parathyroid gland in a cat. × 150.

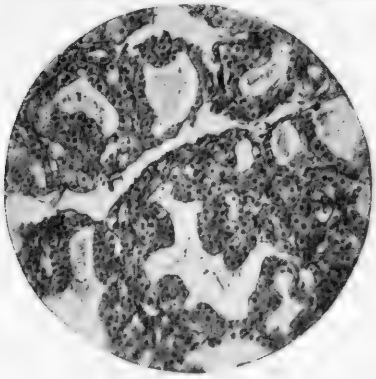

FIG. 1.

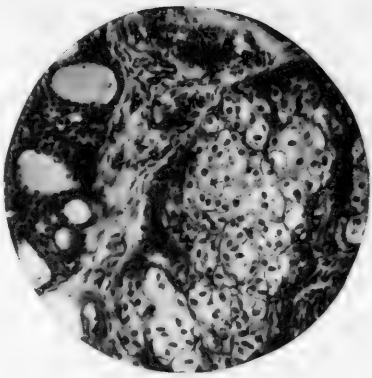

FIG. 4.

FIG. 2.

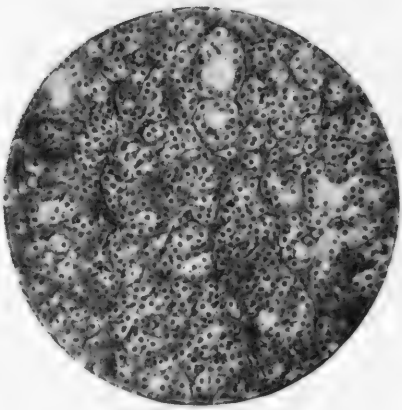

FIG. 5.

FIG. 3.

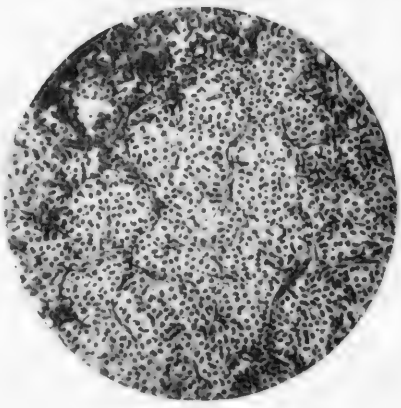

FIG. 6.

(Tanberg: Thyroid and Parathyroid Glands.)

STUDIES ON TREPONEMA PALLIDUM AND SYPHILIS.

V. Further Studies on the Relation of Culture Pallida to Virulent Pallida and on Reinfection Phenomena.

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The writers have already reported studies[1] on immune sera developed in rabbits and sheep by repeated injection of treponemata cultivated from the third generation of syphilitic rabbit orchitis. Although developing powerful agglutinating and treponemicidal powers for the culture *pallida*, these sera had practically no influence on virulent treponemata obtained from lesions, and had no protective value when allowed to act on virulent treponemata before inoculation into rabbits. These studies might be interpreted as indicating one of two things. Either they show that the culture *pallida* are not identical with the virulent *pallida* and that we did not have in our hands a true *Treponema pallidum*, or that during cultivation the *pallidum* had been so changed that it had lost not only its virulence, but certain protective attributes which in the virulent state preserved it from reaction with the serum. The former possibility, though logic and the desire for the omission of no possible factor force its consideration, is not likely for a number of reasons. In the first place, the culture treponema which was used was obtained after passing for three generations through rabbits, a period sufficiently removed from the human lesion to preclude the likelihood of having obtained an organism accompanying the *pallidum* in the human lesion. Furthermore, this organism, as will be shown, is in every respect identical

[1] Zinsser, H., Hopkins, J. G., and McBurney, M., *J. Exp. Med.*, 1915, xxi, 576; 1916, xxiii, 323, 341.

with the strains of *Treponema pallidum* isolated and placed at our disposal by Dr. Noguchi, and the sera which acted powerfully on this strain did so likewise for the Noguchi strains. Thus, this strain of culture *pallidum* is identical with the Noguchi strains, and these, although similarly deprived of virulence and having become subject to serum action, have in former experiments by Dr. Noguchi[2] been shown capable in their early generations of producing typical syphilitic lesions in the testes of rabbits. It has seemed more than likely to us, therefore, that the difference between the virulent and the culture treponemata was due to the fact that in its fully virulent condition, *Treponema pallidum* is in some way insulated from the defensive mechanism of the animal body. This may be due to protective structures analogous to the bacterial capsule, although we were unable by the Porges method of applying acid and heat, or by using sodium oleate as suggested by Lamar in the case of pneumococci, to render these treponemata serum-susceptible. It may, on the other hand, be due to the close biological adaptation of the virulent organisms to the animal body, whereby no reaction between the two takes place. In that case, the failure of susceptibility to antisera produced with non-virulent culture treponemata would imply a rather profound chemical change accompanying the adaptation to culture and the loss of parasitism. However this may be, the fact remains that with the sera so far produced by us, we have not been able to exert any influence on the virulent *pallida*, although the sera we have had have agglutinated the various cultures in dilutions up to 1:2,000 to 1:4,000. We have not yet been in the position to work with horses and to produce sera of highest possible potency.

In the present communication we wish to discuss further experiments bearing upon the problem of the differences between the virulent and culture treponemata, the interrelationship of various culture treponemata, and to report experiments aiming toward further comprehension of the problems of immunity in syphilis.

[2] Noguchi, H., *J. Exp. Med.*, 1911, xiv, 99.

Group Agglutination of Treponemata.

Incidental to the general plan of our studies, we desired to determine the specificity of the agglutination of culture treponemata in order to find out whether the sera might be used for species identification. The immunization of rabbits with culture treponemata is not a simple matter inasmuch as many rabbits die during the process. It seemed at times as though the cultures were toxic, but we have not yet been able to determine definitely whether the accidents of death were due to a true toxicity or to a slow anaphylactic poisoning such as we have noticed in cases of animals treated with many bacteria, a problem upon which we hope to report in another communication. Rabbits were treated with suspensions of culture *pallidum* washed with salt solution and heated to 56°C. for half an hour. In the early experiments we used cultures made in sheep serum rabbit kidney broth under oil; in later experiments we employed young growths obtained without animal tissue, upon coagulated egg medium with ascitic broth, a method devised in this laboratory by Miss Ruth Gilbert and referred to in a previous paper. In all cases, we need hardly add, care was taken to carry out the final serum reactions against cultures grown on animal protein different from that in the cultures with which the animals were immunized, in order to avoid error by protein-antiprotein reactions. The following experiment represents a type of the results obtained by group agglutination experiments (Table I).

TABLE I.

Experiment with Serum 624.

Treponema agglutinated.	Titer of agglutination.
T. pallidum A (homologous)	1 : 2,000
" " (Noguchi 1)	1 : 400
" " (" 2)	1 : 1,000
" *calligyrum*	1 : 4,000
" *refringens*	1 : 200
" *microdentium*	1 : 1,000
" *mucosum*	Agglutinated spontaneously in salt solution controls.

The cultures here employed, apart from *pallidum* A, are cultures placed at our disposal by Dr. Noguchi, and used in these experiments because we could be absolutely sure of their source and of their being true representatives of their respective species, being, in fact, the cultures from which these species were first described by their discoverer. The differences which seem to exist between the three culture *pallidum* strains do not seem to us to mean very much, since minor variations in the character of suspension of each strain used will often change the result to a moderate extent. The treponemata are long and often tangled, and differences almost as great as those noticed above may be due to factors not connected with the specificity

TABLE II.

Absorption of Agglutinins.

Preliminary Titration of a Rabbit Immunized with Treponema pallidum. Strain A.

Suspension of	1:10	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000	Salt.
T. pallidum A.....	+	+	+	+	+	+	+	0	0
" *calligyrum*.....	+	+	+	+	+	+	+	0	0
" *refringens*.....	+	+	+	+	0	0	0	0	0
" *mucosum*.....	0	0	0	0	0	0	0	0	0
" *microdentium*.....	+	+	+	+	+	0	0	0	0
Tonsil organism.....	+	+	+	+	0	0	0	0	0

Agglutination in the Serum of the Same Rabbit (1:50) after Absorption with the Various Strains.

Tested against serum (1:50) absorbed with	Tested against suspension of			
	T. pallidum.	*T. calligyrum.*	*T. refringens.*	*T. microdentium.*
T. pallidum A.....	0	0	0	0
" *calligyrum*.....	0	0	0	0
" *refringens*.....	+	+	0	+
" *mucosum*.....	+	+		
" *microdentium*.....	+	+	+	0
Tonsil organism.....	+	+	+	+
Serum untreated.....	+	+	+	+
Salt solution.....	0	0	0	0

of the serum. For instance, in another experiment in which other suspensions of A and Noguchi 2 were used against the same serum, A, though showing a prezone, went only to 1:500, whereas Noguchi 2 went to 1:1,000. Moreover, experiments reported by us in a previous communication, have also brought out the similarity between the strains. Absorption experiments such as the ones reported here confirm the above (Table II). The close relation between the *pallidum* and the *calligyrum* is shown here.

From the preceding experiments the general impression is gained that the various strains of treponemata are closely related to each other. These observations correspond with cultural and other comparisons which are being carried out by Miss Gilbert with various strains of treponemata from many sources, studied and in part isolated by her. This work is not yet completed and will be reported in a subsequent paper. However, we believe that the work we have done indicates a close group relationship between the various microorganisms.

Action of the Serum of Syphilitic Animals and Man on Culture Pallida.

In studying the immunological problems of syphilis, one of the first hopes fostered is that of eventual success in utilizing the treponema cultures for diagnostic and therapeutic purposes. Our hope of influencing the disease in rabbits by passive immunization with culture antisera, had, of course, been indefinitely deferred by the failure of such sera to act upon or protect against the virulent organisms. However, it still seemed important to determine whether the sera of syphilitic animals and man would possess agglutinating power for the cultures. The ease and speed with which the cultures grow on the egg media might, then, supply a simple method of diagnosis, possibly analogous to the Widal reaction in typhoid. In fact, Kissmeyer[3] has recently made this claim and has expressed the belief that the method would prove of practical diagnostic value.

We may state incidentally that up to the present time, we have not found the sera of syphilitic human beings or animals to exert a considerable agglutinating or immobilizing effect upon virulent trepo-

[3] Kissmeyer, A., *Deutsch. med. Woch.*, 1915, xli, 306.

nemata from rabbits. On one or two occasions, slight differences in this respect between the sera of normal and of syphilitic animals have been noticed, but up to the present time these have been too insignificant to be convincing, and this matter will have to be studied further.

To return to the agglutination of culture organisms by the sera of infected animals and man, we cite the following experiments (Table III).

TABLE III.

Agglutination of Treponema pallidum in Rabbit Sera.

Culture Strains.

A. Normal Rabbit Sera.

1:10	1:25	1:50	1:100	1:1,000
+	0	0	0	Not tested.
+++	+	0	0	" "
0	0	0	0	" "
+++	0	0	0	" "
+++	0	0	0	" "

B. Syphilitic Rabbit Sera.

Time since first appearance of syphilitic lesion.	1:10	1:25	1:50	1:100	1:1,000
11 days	+++	++	0	0	Not tested.
27 "	+++	0	0	0	" "
19 "	+++	+	0	0	" "
2 wks.	++	++	+	0	" "
11 mos.	+++	+	0	0	" "
11 "	++	++	+	0	" "

C. Serum of Rabbit Immunized with Culture Pallida.

1:10	1:25	1:50	1:100	1:1,000
+++	+++	+++	+++	+++

It appears from this experiment and others, similar in result, that the agglutination of the culture *pallida* by the sera of syphilitic rabbits is more regular and slightly higher than that with the sera of

normal rabbits. However, circulating antibody formation is, if it exists at all, very slight, and certainly on the basis of the experiments that we have done so far, we would not venture to distinguish a syphilitic rabbit from the normal rabbit, except in the form of a conjecture, on the basis of the agglutination of the culture organisms.

The absence of antibodies in demonstrable amounts from the sera of syphilitic rabbits may be due to the fact that, in adult rabbits, generalization of syphilis is irregular and incomplete. However, we have so far been unsuccessful in producing agglutinins for the culture *pallida* by the intravenous injection of killed virulent treponemata. The virulent treponemata from lesions used in immunization were obtained by methods which we have previously described, and were killed by heating to 56°C. Two rabbits which received six injections each over a period of 7 weeks, showed no increase of agglutinins for the culture *pallida* as compared with a normal control. A third rabbit was more thoroughly treated. It received sixteen injections over a period of 10 weeks. Its serum compared with two normal controls showed apparently a slight increase in agglutinins (Table IV).

TABLE IV.

Agglutination of Culture Treponemata in the Serum of a Rabbit Immunized with a Virulent Organism.

	1:2	1:10	1:20	1:50	1:100	Control (salt solution).
Immune	+++	+++	+++	++	++	0
Normal 1	+++	++	++	+	+	
" 2	+++	+++	++	+	+	

The suspension here employed was agglutinated more readily than some other suspensions. This variation is one often noted by us and is due to peculiarities of the individual suspension, the cause for which is not entirely clear to us at present. At any rate, it is seen that the difference between the agglutination in the normal sera and in that of the rabbit immunized with dead virulent organisms is a slight one only. We are continuing experiments with sera produced in this way, but have mentioned this in passing since the observa-

tions indicate that the absence of agglutinins in syphilitic rabbits is due to the characteristics of the virulent treponemata, characteristics in which they differ from the culture organisms, rather than to the absence of generalization of the disease in these animals.

A series of similar tests has also been carried out with sera from human syphilis. This was done partly in the hope that serum antibodies might be produced in a species in which the disease is typically a systemic infection; also because we were naturally most interested in the possibility of utilizing such reactions for the diagnosis of human lues. The first experiments were done macroscopically on the sera of syphilitics giving positive Wassermann reactions. As controls the sera of normal individuals and of patients suffering from diseases not syphilitic and giving negative Wassermann reactions were used (Table V).

TABLE V.

Macroscopic Agglutinations.

	No agglutination occurred in	Agglutination occurred in serum 1:5 in	Agglutination occurred in serum 1:10 in	Agglutination occurred in serum 1:20 in
Tertiary syphilis, W. R. +, 23 cases.....	13 cases.	2 cases.	5 cases.	3 cases.
Non-syphilitic diseases, W. R. −, 5 cases	5 "	0 "	0 "	0 "
Normal individuals, W. R. −, 4 cases.....	2 "	1 case.	1 case.	0 "

This series seemed encouraging in as far as syphilitic sera agglutinated more regularly than did the normal, or those of the patients with non-syphilitic diseases, and in some of the syphilitic cases the titer was considerably higher than in the normal individuals that agglutinated. However, macroscopic tests we believe to be at the present time unreliable, and in the subsequent work we set up the tests in agglutination tubes, reading them first macroscopically, but confirming these readings by microscopic examination under the dark-field microscope. Such a large number of sera from individuals without syphilis agglutinated the culture treponemata in dilutions of 1:2, that in most of our subsequent experiments we did not include dilutions lower than 1:5. The following table gives the results in cases we have tested (Table VI).

TABLE VI.

Microscopic Agglutinations.

	No agglutination occurred in	Agglutination occurred in serum 1:2 in	Agglutination occurred in serum 1:10 in	Agglutination occurred in serum 1:20 in	Agglutination occurred in serum 1:50 in	Agglutination occurred in serum 1:100 in
Primary syphilis, W. R. +, 6 cases....	3 cases.	1 case.	2 cases.	0 cases.	0 cases.	0 cases.
Secondary syphilis, W. R. +, 18 cases...	8 "	4 cases.	3 "	1 case.	1 case.	1 case.
Tertiary syphilis, W. R. +, 64 cases...	27 "	7 "	23 "	3 cases.	2 cases.	2 cases.
Tertiary syphilis, W. R. −, 2 cases....	1 case.	0 "	0 "	1 case.	0 "	0 "
Congenital syphilis, W. R. +, 4 cases....	0 cases.	1 case.	3 "	0 cases.	0 "	0 "
Non-syphilitic diseases,* W. R. −, 37 cases...	22 "	4 cases.	7 "	1 case.	2 "	1 case.
Normal individuals, 40 cases.....	35 "	4 "	1 case.	0 cases.	0 "	0 cases.

* The diseases showing high agglutination were heterogeneous, including such conditions as arthritis, tuberculosis, gastro-enteritis, glaucoma, etc., and there seemed to be no relation between disease and agglutination.

An analysis of this table yields the following summary. Agglutination in a dilution of 1: 10 or above was obtained in:

Primary syphilis.....	in	2	out of	6	cases,	or	33	per	cent.
Secondary syphilis.....	"	6	" "	18	"	"	33	"	"
Tertiary syphilis.....	"	31	" "	66	"	"	47	"	"
Non-syphilitic diseases.....	"	11	" "	37	"	"	30	"	"
Normal persons.....	"	1	" "	40	"	"	2	"	"

Before we included non-syphilitic diseases in our plan of experimentation we were hopeful of positive results, since a small percentage of normal people agglutinated the culture *pallida* in dilutions as high as 1:10, whereas a considerable percentage of syphilitics, especially of the tertiary stage, gave such agglutinations. However, sera from various other diseased conditions, for reasons not clear to us at present, also agglutinated the culture *pallida* in a percentage not far removed

from that resulting from the syphilitic serum agglutinations. It is not impossible that some of these, because of the group reactions indicated above, may have been due to the presence of foci of spirochete infection either in the throat, teeth, or other locations in these patients. However, this is a mere assumption, and the fact remains that the occurrence of such agglutinations in non-syphilitic sera detracts considerably from any diagnostic value such reactions might have. We believe that our experiments as far as they have gone in this direction, tend to indicate a slightly increased agglutination power of syphilitic serum for the culture *pallidum*. We do not think, however, that, as at present performed, agglutination of culture *pallida* can be claimed to have any diagnostic value. These results in a general way are in harmony with the specific complement fixations obtained by Noguchi,[4] Craig and Nichols,[5] and Kolmer, Williams, and Laubaugh,[6] as well as with work done on the same problem by us by somewhat different methods.[7] The experiments, as a group, seem to indicate that if circulating antibodies for the culture *pallida* are found at all in the course of syphilis, they are in amount so small that they cannot be definitely determined by available methods. As far as our own experiments have gone up to the present time, the same is true for the virulent *Treponema pallidum*.

Attempts to Protect with Culture Pallida.

Although we had unsuccessfully tried and reported experiments in which attempts were made to protect passively with the sera of rabbits immunized with culture treponemata, we thought it worth while to carry out a few experiments in which inoculation was practiced directly on rabbits actively immunized with such cultures. The following represents an experiment of this kind (Table VII).

[4] Noguchi, *J. Am. Med. Assn.*, 1912, lviii, 1163.

[5] Craig, C. F., and Nichols, H. J., *J. Exp. Med.*, 1912, xvi, 336.

[6] Kolmer, J. A., Williams, W. W., and Laubaugh, E. E., *J. Med. Research*, 1913, xxviii, 345.

[7] In a later publication we intend to report extensively on this phase of our work.

Immunization with Culture before Inoculation.

A.

Nov. 14, 1914. Five rabbits were injected with a good suspension of sheep serum kidney cultures heated to 56°C. plus 0.5 per cent phenol. No bacteria present. Good material.

Injections were made intravenously Nov. 14, 21, and 30.

Inoculated Dec. 11 with six controls, with virulent material from a lesion in Rabbit 12.

TABLE VII.

Treated rabbits.	Injections of culture treponemata.				Results.
	Nov. 14.	Nov. 21.	Nov .23.		
	cc.	*cc.*	*cc.*		
1	3	1	1	All inoculated with virulent material, Dec. 11, 1914.	Doubtful.
2	3	1	1		+
3	3	1	1		+
4	3	1	1		Chancres.
5	3	1	1		+
Controls.					
6					+
7					+
8					+
9					+
10					Died in less than a month after inoculation.
11					Died in less than a month after inoculation.

B.

Two rabbits were immunized with dead ascitic culture material, highly concentrated. One of these died in the course of immunization, the other one, No. 13, received twelve injections between Dec. 18 and Feb. 19. On Feb. 23 it was inoculated with virulent material into both testes. On Mar. 6 it developed a lesion in the left testis which was removed for other experiments,[8] and on Mar. 18 it developed a large diffuse lesion in the right.

Since these experiments take a considerable amount of time, and since, in the last case at least, extensive and satisfactory immunization with the culture *pallidum* had been practiced, we feel justified in reporting these few experiments as indicating that intravenous treatment with culture *pallida* is not likely to confer any considerable degree of resistance upon rabbits.

[8] The removal of testes for the obtaining of virulent material for rabbits is always carried out under ether anesthesia.

In the hope that localized immunization might result more favorably, we treated two rabbits intratesticularly with living ascitic egg cultures from Jan. 21, 1916, to Apr. 21, 1916, a total of eight injections being given. On May 3 they were inoculated with virulent treponemata together with two controls. On May 23 one of these rabbits developed lesions on both sides. The other one developed a lesion on June 25. One of the controls only has shown a lesion to date.

Had experiments such as those reported immediately above shown more favorable results, we should feel unjustified in publishing on the basis of so small a series. However, a negative result in an experiment of this kind is far more convincing than a positive one, and, although this work is being continued, we feel at present that it is not likely that either local or general treatment with culture *pallida* will protect.

Incidentally, the last experiment, together with two other rabbits not yet reinoculated, shows that repeated local injection of living culture treponemata will not produce a lesion, and that, as far as the rabbit testis is concerned, our cultures have lost all virulence.

Local and general immunization with suspensions of dead virulent treponemata are going on, but these are slow owing to the difficulty of obtaining material for injection and because of the frequent death of rabbits during the process. We are not yet, therefore, prepared to report on this work. However, the work of Uhlenhuth and Mulzer indicated that vaccines prepared with dead virulent material conferred no protection.

Reinfection Experiments in Rabbits.

The work of Uhlenhuth and Mulzer[9] has given us a thorough knowledge of the conditions prevailing in rabbits that have been inoculated with syphilitic virus. It is well known that in man and in monkeys a resistance is acquired during the course of syphilis which seems to protect against superinfection during a period which begins with, or shortly after, the development of the chancre, lasts throughout the secondary period, and, though waning, through the tertiary. It is also generally held that complete cure reestablishes susceptibility and that true reinfection, though rare, probably because of the relative infrequency of complete cure, is, nevertheless a fact. The extensive tabulations of John[10] may be referred to for confirmation of this statement. In rabbits the conditions are not the same, inasmuch as superinfection and reinoculation have almost invariably been suc-

[9] Uhlenhuth, P., and Mulzer, P., *Arb. k. Gsndhtsamte.*, 1913, xliv, 307.

[10] John, F., *Samml. klin. Vortr.*, 1909, N. F., *Inn. Med.*, No. 157–64, 559.

cessful even during the existence of syphilitic lesions. Bertarelli[11] succeeded in reinoculating the cornea of a rabbit which had been inoculated with syphilis on a previous occasion. Uhlenhuth and Mulzer, Fontana,[12] Neisser,[13] and Pürckhauer,[14] have also found that infections of the eye were possible at a period during which the opposite eye was still syphilitic, the last named authors even showing that the cornea of the same eye could be twice inoculated. Tomasczewski[15] found that the corneal infection did not protect against scrotal infection, and, *vice versa*, although scrotal infection conferred a skin immunity. Ossola[16] and Truffi[17] also found that successful skin inoculations in rabbits made reinfection of the skin difficult although the protection so conferred was not absolute. In estimating the value of the last experiments, it should not be forgotten that successful skin inoculations in rabbits is not a thing to be obtained with regularity.

Uhlenhuth and Mulzer in extensive experiments on rabbits came to the following conclusions. (1) Syphilis of the testis and of the eye in rabbits does not protect against reinfection, and this holds good in all cases whether or not the syphilitic testicular or eye lesions have healed spontaneously, have been cured by specific drugs, or are still existing. (2) The pathological lesions resulting from such reinoculations do not differ from, and are not less severe than those following the first inoculation.

In only two animals did they notice resistance to reinoculation and these were very young rabbits that had been generally syphilized by the intracardial injection of the virus. In immunization experiments, they found that repeated injections of rabbits with the serum of rabbits intravenously treated with virulent material did not protect and that no vaccine produced with luetic material had any effect on subsequent inoculation.

In general, we may subscribe to these results of Uhlenhuth and Mulzer on the basis of our own experience in a large number of experiments. However, in one important particular, our results seem to differ distinctly from the conclusions of Uhlenhuth and Mulzer, and that is in the acquisition of a powerful localized resistance by the particular tissues which have been the seat of a syphilitic lesion at some time. After obtaining the results which we tabulate below (Table VIII), we went over the protocols of Uhlenhuth and Mulzer and found that in a number of cases reported by them, their results bear out our own, although apparently in their summaries, they overlooked the significance of their data.

[11] Bertarelli, E., *Centr. Bakteriol., 1te Abt., Orig.*, 1908, xlvi, 51.

[12] Fontana, A., *Riv. ig. san. pubb.*, 1907, xviii, 646.

[13] Neisser, A., Beiträge zur Pathologie und Therapie der Syphilis, Berlin, 1911, 569; also *Arb. k. Gsndhtsamte.*, 1911, xxxvii, 569.

[14] Pürckhauer, R., *Arb. k. Gsndhtsamte.*, 1911, xxxvii, 576.

[15] Tomasczewski, E., *Berl. klin. Woch.*, 1910, xlvii, 1447.

[16] Ossola, cited by Truffi below.

[17] Truffi, M., *Centr. Bakteriol., 1te Abt., Orig.*, 1909, lii, 555; 1910, liv, 337.

TABLE VIII.

Reinoculation of Rabbits.

Rabbit No.	1st inoculation.	Lesion.		Interval.	2nd inoculation.	Results.
		First appearance.	When healed.			
14	Nov. 6, 1914. Both testes. Strain F.	Jan. 30, 1915. Nodules in both testes.	May 10.	6 mos.	Nov. 30. Both testes. Strain A.	Negative until Jan. 11, 1916. Died.
15	July 8, 1915. Both testes. Strain S.	Aug. 25, Nodules in right testis. Left negative.	Sept. 10.	6 wks.	Nov. 30. Both testes. Strain A.	Testes negative until Feb. 23, 1916. Lost. Jan. 6. Developed keratitis in left. Treponemata found in nasal secretion. Feb. 23. Keratitis gone.
16	Apr. 2, 1915. Both testes. Strain A.	May 2. Right scrotal lesion. Nodule in left testis.	Not examined until Sept. 10. Negative.	At least 6 wks., probably much longer.	Nov. 24. Both testes. Strain A.	Negative until Apr. 10, 1916. Died. No lesions except partial loss of hair on back, Jan. 24.
17	Nov. 21, 1914. Both testes. Strain T.	Dec. 22. Nodule in left testis. Jan. 6, 1915. Diffuse orchitis in right.	Sept. 10, 1915.	At least 6 wks., probably much longer.	Nov. 24. Right testis. Strain A.	Negative until Jan. 18, 1916, when doubtful nodule was noted, probably scar of the old lesion. Jan. 24, negative. Thereafter negative until Apr. 10. Died.
18	July 8, 1915. Both testes. Strain S.	Aug. 28. Nodule in left.	Sept. 10. Gone.	6 wks.	Nov. 24. Both testes. Strain A.	Negative until May 15, 1916. Died.

19	Nov. 10, 1915. Both testes. Strain F.	Nov. 24. Diffuse orchitis, left. Dec. 1. Right and left. Positive to puncture.		Less than 2 wks.	Dec. 18. Both testes. Strain A.	Jan. 12, 1916. Left testicle positive on puncture; unusually large number of spirochetes. Feb. 9. Nodule on both sides. Apr. 25. " " right.
20	Dec. 11, 1914. Intravenously immunized with culture treponemata and inoculated in both testes. Strain A.	Mar. 11, 1915. Nodule in left testis.		About a year.	Mar. 14, 1916. Left testis. Strain A.	Negative until Apr. 19, then very small nodule (left) which showed no treponemata on puncture. Died June 1. Negative.
21	May 26, 1915. Both testes. Strain A.	June 30. Diffuse orchitis in right. Sept. 2. Small nodule in left.	Jan. 24, 1916.	7 wks.	Mar. 14. Both testes. Strain A.	Doubtful nodule on right, Mar. 28. Negative on puncture. Negative within few days, then remained negative (June 20).
22	Sept. 8, 1915. Both testes. Strain L.	Oct. 4. Small nodule in left. Oct. 7. Small nodule in right and left. Nov. 26. Right excised.	Jan. 6, 1916.	2 mos.	Mar. 14. Left testis. Strain A.	Negative until May 29. Died.

TABLE VIII—*Concluded.*

Rabbit No.	1st inoculation.	Lesion.		Interval.	2nd inoculation.	Results.
		First appearance.	When healed.			
23	Oct. 21, 1915. Both testes. Strain F.	Nov. 24. Diffuse orchitis in right and left. Left removed.	Chancre until Feb. 23, 1916. Healed.	3 wks.	Mar. 14. Right testis. Strain A.	Apr. 19. Small nodule on right. Negative on puncture. Negative until June 5. Died.
24	Oct. 21, 1915. Both testes. Stain F.	Nov. 24. Nodule in left. Feb. 23, 1916. Doubtful nodule in right.	Dec. 2. Left healed.	14 wks.	Mar. 14. Both testes. Strain A.	Apr. 11. Died. No lesion.
25	Oct. 4, 1915. Both testes. Strain T.	Nov. 24. Nodule in right. Jan. 28, 1916. Nodule in left. Right excised.	Feb. 9.	5 wks.	Mar. 14. Left testis. Strain A.	Mar. 28. Doubtful small nodule. May 5. Negative on puncture. Remained negative (June 20).
26	June 9, 1915. Both testes. Strain A.	Aug. 25. Nodule in left excised. Jan. 27, 1916. Nodule in right.	Feb. 9.	5 wks.	Mar. 14. Right testis. Strain T.	Apr. 11. Distension of right testis. Negative to puncture. Apr. 23. Doubtful nodule, negative to puncture. May 23. Died. Negative.
27	Nov. 18, 1915. Both testes. Strain A (+ immune serum).	Jan. 4, 1916. Nodules in right and left. Right positive on puncture.	Feb. 23. Right healed.	3 wks.	Mar. 18. Both testes. Strain T.	Apr. 3. Chancre, right and left. Negative on puncture. Apr. 26. Nodules right and left. Negative to puncture. May 1. Died. No lesion.

28	Dec. 2, 1915. Both testes. Strain S.	Dec. 28. Small nodule in left testis. Jan. 4, 1916. Diffuse orchitis (?) in right.	Feb. 9.	5 wks.	Mar. 18. Both testes. Strain T.	Apr. 6. Chancres on right and left testes. Apr. 11. Nodules on right and left. Non-motile treponemata on puncture.
29	Dec. 6, 1915. Both testes. Strain T.	Dec. 28. Nodules in right and left. Jan. 8, 1916. Right removed.	Jan. 27.	7 wks.	Mar. 18. Left testis. Strain T.	Apr. 26. Doubtful nodule, left. Negative on puncture May 2. Negative (?) May 5. Negative on puncture.
30	Aug. 4, 1915. Both testes. Strain F.	Sept. 8. Diffuse orchitis in right and left. Right excised.	Nov. 4.	19 wks.	Mar. 18. Left testis. Strain T.	May 23. Nodule (left); many motionless treponemata.

Rabbits 14 and 15 were controlled by Rabbit 31, inoculated with the same material, which developed bilateral lesions.

Rabbits 16, 17, and 18 were controlled by Rabbit 32, inoculated with the same material, which showed bilateral lesions.

Rabbits 20, 21 22, 23, 24, and 25, were controlled by Rabbit 33, which showed a positive lesion on one side.

Rabbits 26, 27, 28, 29, and 30, were controlled by Rabbit 34, which showed bilateral lesions.

During the period of reinoculation of these rabbits (Nov., 1915, to Mar., 1916), 30 rabbits were inoculated for other purposes by the same technique. These showed 97 per cent positive results, 80 per cent of the rabbits developing bilateral lesions. In contrast to this, the above group of 17 reinoculated rabbits showed in 1, or 6 per cent, typical lesions; in 2, or 12 per cent, small lesions positive to puncture, in 8, or 47 per cent, small doubtful nodules, negative to puncture and probably never syphilitic in nature; and 6, or 35 per cent, were absolutely negative.

The protocols of Uhlenhuth and Mulzer to which we have referred[18] deal with a series of eleven rabbits inoculated in the testis and reinoculated after apparent recovery from first infection. Of this series, three, inoculated for the first time on one side only, were negative after the second inoculation. Two were inoculated first in the right, and then in the left from the lesion developed in the right testis, and therefore have no bearing on our experiments. Two were inoculated in both testes, but developed lesions only on the left. On reinoculation into both testes they developed lesions only on the right. One gave no response to the first inoculation but developed lesions on both sides after the second.

Only three out of the eleven developed lesions on reinoculation on the same side as that on which the first lesion had appeared. One of these showed a diffuse orchitis after the first inoculation but only a localized sac lesion on reinoculation. Another, although reinoculated on the left side only, subsequently developed lesions on both sides, indicating that the original infection had not yet run its course. The third, which developed diffuse bilateral lesions after the first inoculation, and a diffuse lesion on the left after the second inoculation, is the only one of the series which seems to have developed a second satisfactory lesion on reinoculation at the site of the original disease.

Thus, in a general way, although not so interpreted by them, the results of Uhlenhuth and Mulzer are not out of harmony with the results obtained by us in the seventeen rabbits reported above. Of these seventeen reinoculations, twelve failed to show lesions in testes which had shown lesions before. Another one (No. 20) developed a small nodule which was negative to puncture, but we shall not include this with the twelve as entirely negative, as we wish to be particularly cautious. The occasional small nodules which developed in the testis and were negative to puncture, in two or three of these rabbits, even if they had proved to be syphilitic by successful puncture, would indicate a considerably less extensive lesion than is ordinarily found in such inoculations, and at present we rarely fail to obtain spirochetes on puncture in syphilitic rabbits. Rabbit 27 developed chancrous lesions of the skin, therefore histologically not on the site of the previous lesion of the testis, and in this case, no treponemata could be found. Rabbit 28 undoubtedly developed small lesions positive to puncture, although the treponemata found were non-motile, an occurrence which we cannot at present satisfactorily explain. But in this rabbit, the first inoculation products were doubtful. No. 30 developed a small nodule in a testis which had

[18] Uhlenhuth and Mulzer, *Arb. k. Gsndhtsamte.*, 1913, xliv, 453.

previously been the seat of a diffuse orchitis. But the lesion was small and limited in extent and the treponemata found on puncture were motionless. One rabbit in this series (No. 19), although exhibiting definite lesions on the first inoculation, developed frank lesions after the second inoculation. It will be noticed that this is the only rabbit in which reinoculation was undertaken less than 2 weeks after apparent healing of the first lesion.

If we analyze these results together with the few data gathered from the paper of Uhlenhuth and Mulzer, they seem to us to furnish evidence that there is a distinct local resistance to reinfection developed at the site of a previous lesion. In rabbits the conditions are peculiarly favorable for experiments bearing on this point. Although generalization of the *pallida* probably occurs fairly regularly in very young intracardially inoculated rabbits, and to a limited extent in intratesticularly inoculated adults, the treponemata do not easily form lesions on other sites or active pathological reactions in many of the organs of rabbits. It is known of these animals as we have stated above that the existence of a lesion in one testis does not appear to confer resistance in other parts of the body, and we cannot speak in rabbits as we can in human beings and monkeys, of resistance to superinfection coincident with the existence of the disease. Since the experiments cited above, then, seem to point out that this resistance is conferred upon the site of pathological reaction, we are inclined to deduce that resistance to syphilis is a localized process which affects the tissues in which the reaction to the treponemata has taken place, and is not a property conferred, as in resistance to some bacterial infections, by a diffusion of antigen and a consequent production of sessile and circulating antibodies in parts remote from the point of actual infection. This complements our results and those of others who have failed to find any considerable amount of circulating antibodies, and would explain the universal failure to produce immunization either by treatment with dead syphilitic virus or, passively, with the sera of syphilitic or vaccinated human beings and animals.

The difficulty which arises in interpreting these experiments lies in determining whether the testes, after reinoculation, still harbored spirochetes from the first inoculation (the reinoculation being really

a superinfection), or whether they had completely recovered. It is practically impossible to determine this, since negative puncture is insufficient to permit conclusions as to the absence of treponemata from the entire testis. However, in some of our rabbits, 2 to 3 or 6 months, or nearly a year had elapsed, and to ordinary examinations the testis seemed normal at the time of reinoculation. We believe that the reaction capacity of the tissue cells must be the important element and not the possible persistence of a limited number of treponemata which are no longer capable of inducing reaction. As far as the analysis of the immunological condition is concerned, it is, after all, of chief importance whether the treponemata persisting or newly introduced can or cannot cause pathological injury.

CONCLUSIONS.

1. Immune sera produced in rabbits by treatment with our Culture Strain A of *Treponema pallidum* agglutinated not only the homologous strain, but also the Noguchi strains, and indicate a close group relationship of other non-pathogenic treponemata. Absorption experiments confirmed this, indicating a close relationship between the *pallidum* and the *calligyrum*.

2. Culture treponemata are not agglutinated to a much greater extent by the sera of syphilitic rabbits than they are by those of normal rabbits.

3. Culture treponemata are not agglutinated to any considerable extent by the sera of rabbits immunized with virulent treponemata.

4. The sera of syphilitic patients, especially those in the tertiary stages, agglutinate culture *pallidum* to a slightly greater extent than do those of normal individuals, but the culture *pallidum* is agglutinated to an almost equal degree by the sera of many individuals with diseases other than syphilis. We do not think that we could definitely distinguish the syphilitic from the non-syphilitic serum by the agglutination of the culture *pallida*, and therefore we do not believe that the reaction has any diagnostic value at present.

5. Immunization with culture *pallidum*, either general or local, does not seem to confer upon rabbits any considerable degree of resistance to inoculation with virulent treponemata.

6. Rabbits that have once exhibited lesions in the testis are not easily reinfected at the same site if reinoculation is practiced more than a month or so after apparent healing of the lesion. We believe that the experiments above recorded strongly suggest that resistance to syphilis in rabbits is a localized cell phenomenon not dependent upon a generalized reaction on the part of tissues remote from the site directly involved in reaction with the invading treponemata. Antibodies analogous to those formed in most bacterial infections appear in the general circulation in slight amount, if at all. The finding of many motionless treponemata in a few of the small lesions following reinoculation suggests the possibility of a purely localized formation of antibodies. This was expressed by Landsteiner some years ago when he spoke of the localized formation of agglutinins when they were absent in the general circulation.

We hesitate to apply these results too generally to the conditions prevailing in human syphilis, but they contain the possibility of an explanation for the apparent skin immunity of the secondary period, and the later successive involvement of some organs and tissues when others remain normal and when external superinfection is successfully resisted.

THE RESPIRATORY MECHANISM IN PNEUMONIA.*

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Our first investigations established the facts that the blood pressure and the vasoreflex[1] mechanism are not, as a rule, seriously impaired in pneumonia and that hearts from dogs dead of this disease contract normally when supplied with normal blood.[2] In the course of these experiments it was often observed that the respiration failed before the circulation; if artificial respiration was provided, the heart would continue to beat sometimes an hour or more after spontaneous breathing had failed. We therefore resolved to compare the condition of the respiratory mechanism in healthy cats and dogs with its condition in animals infected with pneumonia.

* Earlier papers in these studies of pneumonia are as follows: Newburgh, L. H., and Minot, G. R., *Arch. Int. Med.*, 1914, xiv, 48. Porter, W. T., and Newburgh, L. H., *Boston Med. and Surg. J.*, 1914, clxx, 125. Means, J. H., Newburgh, L. H., and Porter, W. T., *Boston Med. and Surg. J.*, 1915, clxxiii, 742; 1916, clxxiv, 464. Porter, W. T., Newburgh, L. H., and Newburgh, I., *Am. J. Physiol.*, 1914, xxxv, 1. Newburgh and Porter, *J. Exp. Med.*, 1915, xxii, 123. The first study cited consisted of measurements of the blood pressure in patients with pneumonia by Dr. Newburgh and Dr. Minot in the wards of the Massachusetts General Hospital. The remaining studies are of experimental pneumonia. These investigations, as well as the present research, were made in the Laboratory of Comparative Physiology of the Harvard Medical School, and under its direction. The cost of the present research was met in part by a grant from the Proctor Gift, contributed by the Department of Medicine of the Harvard Medical School.

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[1] Porter, Newburgh, and Newburgh, *Am. J. Physiol.*, 1914, xxxv, 1.

[2] Newburgh, and Porter, *J. Exp. Med.*, 1915, xxii, 123.

A method devised by Haldane and his associates may be used for this purpose. When an animal breathes through a closed system of tubes, the carbon dioxide in the inspired air progressively increases. In normal animals, this increase in the carbon dioxide stimulates the respiratory center and causes a corresponding increase in the volume of the air passing in and out of the chest. Thus, in cats and dogs, when the carbon dioxide rises to 3 per cent, the volume of the air inspired is usually doubled. The condition of the respiratory mechanism may be measured by the volume of air breathed per minute as the carbon dioxide in the inspired air rises from 1 to 5 per cent.

It will be shown in this investigation that the reaction of the respiratory mechanism to carbon dioxide is greatly diminished in pneumonia.

Method.

Cats and dogs were employed, and ether was given in all procedures that might cause pain.

The organism used was the *Bacillus pneumoniæ* of Friedländer obtained from the stock culture in the Bacteriological Laboratory[3] of the Harvard Medical School through the kindness of Dr. Sisson. This culture had been used for experimental pneumonia for 18 months or more. It was isolated originally by Dr. A. E. Steele from a patient in the Massachusetts General Hospital. The organism was passed through three guinea pigs to increase the virulence to such a degree that 1 cc. of a broth culture injected into the peritoneum killed a guinea pig in 12 hours. The virulence was maintained at this point by occasional passage through additional guinea pigs. Some months after beginning the research, the stock culture was rejuvenated by a bacillus from a case of Friedländer's septicemia, for which we are indebted to Dr. Steele.

Pneumonia was produced by injecting into the trachea broth cultures incubated from 18 to 24 hours. The quantity injected varied;

[3] We are indebted to Dr. Calvin C. Page for the bacteriological examinations of the culture. He found that the culture corresponded morphologically and biologically with published descriptions of *B. pneumoniæ*. Nine different sugars were used for fermentation. The two strains fermented the same sugars, but the amount and time of maximum gas formation varied slightly.

we found that 2 cc. per kilo of body weight caused death in dogs usually in about 30 hours. The dogs were given subcutaneously from 0.5 to 1.0 per cent of a 3 per cent solution of morphine sulphate, to lessen or prevent coughing. The culture was injected after the method of Lamar and Meltzer.[4] In cats, owing to the small size of the bronchus, the injection was made into the trachea near the bifurcation.[5]

The course of the disease may be seen from the following protocols.

Dog 9.—Nov. 28, 1915. 11.30 a.m. Inoculated.

Nov. 29, 9 a. m. Rectal temperature 39°C. Breathing labored and rapid. Walks about easily. Restless.

4 p.m. Temperature 39°C.

6 p.m. Temperature 37°C. Unsteady.

7.30 p.m. Temperature 36°C. Lies on one side. In coma.

8.10 p.m. Convulsion. Breathing stops. Artificial respiration.

8.45 p.m. Apex beat of heart can still be seen. No corneal reflex.

9.30 p.m. Heart beat ceases.

Dog 14.—Dec. 10, 1915. 10 a.m. Inoculated.

Dec. 11, 9 a.m. Temperature 40°C. Respiration rapid and labored. Can stand, but prefers lying down. Restless.

10.30 a.m. Temperature 39°C.

1.30 p.m. Temperature 38.2°C. Able to stand.

3.00 p.m. Temperature 38°C. Cannot stand or sit up.

4.10 p.m. Temperature 37°C. Coma.

6.45 p.m. Temperature 35°C. Corneal reflex present.

8.20 p.m. Temperature 35°C. Corneal reflex absent.

8.30 p.m. Death.

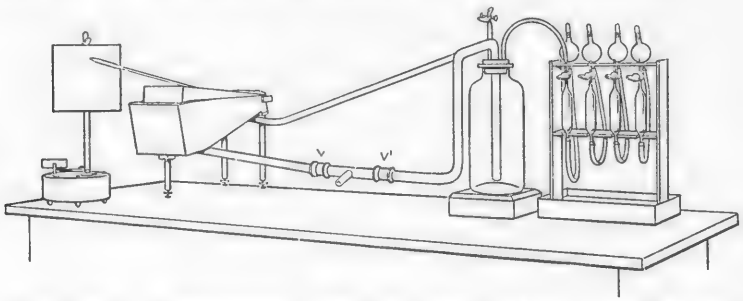
Bacillus pneumoniæ, used as we have described, is practically always fatal. The fatal issue is almost invariably preceded by a characteristic fall in temperature. This fall is so constant as to form an admirable prognostic sign, of much value when it is desired to measure the respiratory reaction shortly before death. Thus it is possible to separate the animals into two groups, those whose tem-

[4] Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 135.

[5] As far as we are aware, acute pneumonia in cats was produced first by Porter and Newburgh (*Boston Med. and Surg. J.*, 1914, clxx, 125). It was produced in dogs by Kinjoun and Rosenau (*Bull. Hyg. Lab., U. S. P. H.*, 1897, 762) and by Lamar and Meltzer (*J. Exp. Med.*, 1912, xv, 133).

perature is above 37.5°C. and those below this level. The latter animals were always near death.

The measurements to be presented in this investigation were made only on cats and dogs which afterwards died of pneumonia, and in which an autopsy showed the presence of typical consolidation.

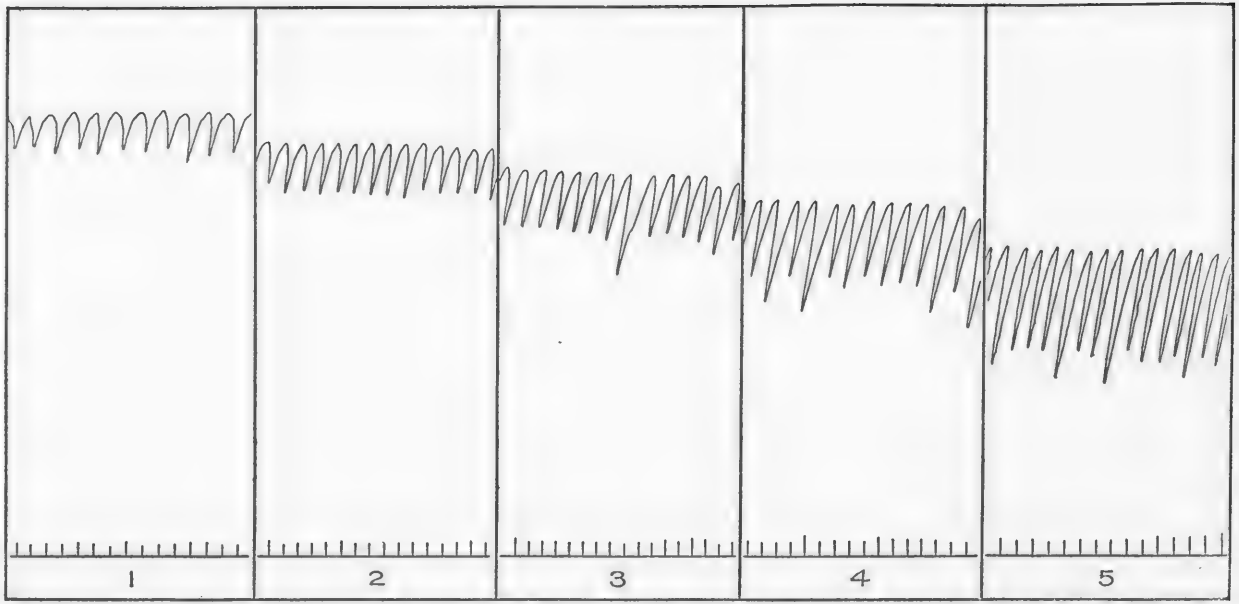


TEXT-FIG. 1. The apparatus. From left to right: the kymograph; the spirometer; the Tissot valves, *v*, *v'*; the bottle; the mercury sample tubes. The electromagnetic time signal and the Haldane apparatus are not shown.

A portion of the apparatus employed is shown in Text-fig. 1. The tracheal cannula of the animal in which the respiratory reaction was to be measured was joined to a rubber tube placed between two Tissot[6] valves connected in such a way that the animal breathed into a spirometer[7] and out of a bottle connected in its turn with the spirometer, so that the lungs, the spirometer, the bottle, and the connecting tubes formed a closed system. Evidently by this arrangement the volume of the air passing into and out of the chest was recorded by the spirometer, while the carbon dioxide exhaled by the animal constantly accumulated in the closed system. Samples of the air thus enriched with carbon dioxide were withdrawn at frequent intervals by the mercury tubes shown in Text-fig. 1. As each sample was taken, a mark was made on the spirometer record above the time record (the electric time signal is not shown in Text-fig. 1). As the carbon dioxide in the respired air increased, the spirometer curve became deeper, until the maximum reaction was reached (Text-fig. 2).

[6] Metal flap valves so lightly constructed as not to impede respiration.

[7] This quantitative aeroplethysmograph was invented by Professor J. Gad.



TEXT-FIG. 2. Apr. 13, 1916. Volume of tidal air in a normal dog breathing 1, 2, 3, 4, and 5 per cent of carbon dioxide. The time curve marks alternate seconds. The gradual descent of the curve is due to the gradual diminution of oxygen in the closed system. This dog was normal save for a tracheotomy tube; it was trained to stand quietly during the experiment. In Text-fig. 3, this curve is plotted with the curve of a normal decerebrate dog.

The spirometer was empirically graduated by recording the vertical rise of its writing point with each increase of 50 cc. in its contents. When the experiment was finished, the empirical graduation scale was used to measure the number of cubic centimeters breathed in the minute nearest the mark at which the air sample was withdrawn. The air in the sample was analyzed for carbon dioxide in a Haldane apparatus and the percentage written against the ventilation (Table I). Thus the cubic centimeters breathed per minute as the carbon

TABLE I.

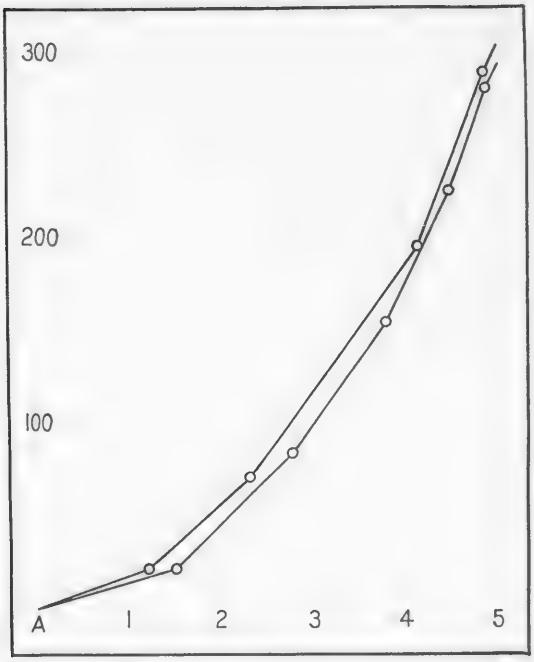
The Reaction of the Respiratory Mechanism to Carbon Dioxide in the Normal Decerebrate Dog (No. 2 A).

Nov. 19, 1915.

Carbon dioxide in the inspired air.	Respirations per min.	Volume of each respiration.	Tidal air per min.	Percentile increase in tidal air.
per cent		*cc.*	*cc.*	*per cent*
Room air.	26.5	200	5,300	
1.50	30.0	215	6,450	21
2.74	35.0	280	9,800	85
3.73	36.0	375	13,500	155
4.46	38.0	450	17,100	223
4.86	45.0	450	20,220	282
5.28	48.0	465	22,350	320

dioxide rose from the atmospheric level to 5 per cent or more were recorded accurately. When plotted on coordinate paper after the manner of Peabody,[8] as in Text-fig. 3, a curve results, expressing the reaction of the respiratory mechanism to increasing quantities of carbon dioxide. Upon this curve may be read the cubic centimeters breathed per minute at 1, 2, 3, 4, and 5 per cent of carbon dioxide. The data for Table II and Tables IV to XI were obtained in this way.

[8] Peabody, F. W., *Arch. Int. Med.*, 1915, xvi, 851.



TEXT-FIG. 3. Volume of tidal air in two normal dogs; the upper curve without decerebration; the lower with decerebration. The data for the decerebrate curve are given in Table I. The numbers at the left of the figure give the percentile increase in tidal air; the numbers below give the per cent of carbon dioxide.

TABLE II.

The Effect of Ether upon the Respiratory Reaction to Carbon Dioxide in the Dog.
Apr. 13, 1916.

Carbon dioxide in the inspired air.	Percentile increase in tidal air per min.	
	20 min. after ether.	35 min. after ether.
per cent	*per cent*	*per cent*
1	15	18
2	28	56
3	50	118
4	102	190
5	152	302

In certain dogs, ether was used for some operation preliminary to the carbon dioxide test. Ether lowers the irritability of the respiratory mechanism. Thus in the experiment of April 13, 1916, a dog weighing 4.5 kilos was etherized and tracheotomized at 10.45 a.m. At 11.05 and at 11.20 a.m. the carbon dioxide reactions were measured. They were as shown in Table II. The depression of the respiratory mechanism from ether is very evident. Where ether is used, time must be allowed for its elimination.

It need hardly be said that before each experiment the spirometer, bottle, and connecting tubes were washed free of carbon dioxide, except that in room air. Since the method consists of a comparison of the volume breathed per minute in atmospheric air with that breathed when the air contains increasing quantities of carbon dioxide, it is important that the initial or atmospheric ventilation should be accurately recorded, and the animal must therefore be kept quiet. Ether is, of course, excluded, as we have already pointed out. It is known that morphine and chloral also depress the respiratory center. Urethane cannot be used, as is shown by the experiment recorded in Table III. It appears from Table III that with urethane the

TABLE III.

The Effect of Urethane upon the Respiratory Reaction to Carbon Dioxide in the Cat. Sept. 15, 1915.

Carbon dioxide in the inspired air.	Respirations per min.	Volume of each respiration.	Tidal air per min.	Percentile increase in tidal air.
per cent		*cc.*	*cc.*	*per cent*
Room air.	52	19	987	
0.60	50	22	1,100	11
2.05	48	26	1,249	26
3.41	50	32	1,600	62
4.45	53	35	1,855	88
5.18	55	41	2,255	128
5.94	59	48	2,830	187

cubic centimeters breathed per minute when the carbon dioxide in the inspired air was 5 per cent had increased in the urethane cat only about 120 per cent, whereas, reference to Table VIII will show that the normal increase should be about 285 per cent.

Not being able to keep certain animals absolutely quiet with a drug that would not depress the respiratory center, we resorted in these cases to decerebration under ether anesthesia. The pons was divided by a seeker sufficiently blunt to prevent injury to the blood vessels. Time was allowed for elimination of the ether necessary to produce narcosis. Both the normal and the pneumonic cats were decerebrated. The normal dogs were also decerebrated, but the pneumonic dogs were measured after the disease had made them lethargic or comatose.

All the animals in which the respiratory function seemed injured by decerebration were excluded. As a rule, the respiration and circulation are wholly normal after decerebration. The animals often lived many hours after this operation. Cats lived several days. Nevertheless, it seemed desirable to make sure by a quantitative test that decerebration did not depress the respiratory mechanism. An additional reason was that no one, as far as we are aware, had previously measured the carbon dioxide reaction in a normal or in a decerebrate dog. On April 13, 1916, we accordingly measured the carbon dioxide reaction in a dog wholly normal except for a tracheotomy tube. The increases in tidal air as the inspired carbon dioxide rose from 1 to 5 per cent were 18, 56, 118, 190, and 302 per cent, respectively. These are compared in Table IV with the averages

TABLE IV.

The Respiratory Reaction to Carbon Dioxide in a Normal Dog (Tracheotomy Only) and in Normal Decerebrate Dogs.

Carbon dioxide in the inspired air.	Percentile increase of tidal air in a normal dog.	Average percentile increase of tidal air in decerebrate dogs.
per cent	*per cent*	*per cent*
1	18	19
2	56	48
3	118	96
4	190	182
5	302	285

obtained from normal decerebrate dogs. It is obvious that decerebration does not impair the reaction of the respiratory mechanism to carbon dioxide.

We pass now to a general criticism which at first seems important but which upon reflection is easily proved fallacious. Since the volume of the tidal air in animals with pneumonia is often greater than the tidal air in normal animals, it might be supposed that pneumonia animals do not increase their respiration when stimulated by carbon dioxide because they are already breathing as much as they can. That this is not a source of error is demonstrated by comparing the average carbon dioxide reaction of animals whose tidal air at the beginning of the observation has in every case approximately the same volume. When the carbon dioxide in the inspired air was 2 per cent:

4 normal cats breathed.....1,233 cc. per min.
4 pneumonia cats breathed.....1,232 " " "

Thus the tidal air in both groups was practically equal in volume.

When the carbon dioxide in the inspired air rose to 4 per cent, the same

4 normal cats breathed.....2,330 cc. per min.

but the

4 pneumonia cats breathed.....1,758 " " "

Thus at 2 per cent the pneumonia cats and the normal cats breathed alike, while at 4 per cent the pneumonia cats had increased less than half as much as the normal cats.

The detailed observations of the four normal and four pneumonic cats are set forth in Table V.

TABLE V.

Pneumonic Cats and Normal Cats May Breathe Practically the Same Volume of Air per Minute at the Beginning of the Test; but when the Inspired Carbon Dioxide Rises, the Pneumonic Ventilation Increases Much Less than the Normal; the same is true of Pnuemonic Dogs.

No. of animal.	Percentile increase in cc. breathed per min. as the inspired carbon dioxide increases from 1 to 4 per cent.			
	1 per cent.	2 per cent.	3 per cent.	4 per cent.
Pneumonic cats.	*cc.*	*cc.*	*cc.*	*cc.*
18 B	1,160	1,350	2,020	2,780
22	1,190	1,295	1,430	1,490
23 C	1,070	1,165	1,345	1,720
25*	1,070	1,080	1,090	1,040
Average.....	1,123	1,223	1,471	1,758
Normal cats.				
1 A	1,080	1,360	1,930	2,450
5 B	1,045	1,150	1,250	1,620
6 C	960	1,180	2,020	2,950
15 A	1,005	1,240	1,680	2,300
Average.....	1,023	1,233	1,720	2,330

* Cat 25 died at the close of this experiment; the observation was excluded from our final results (Table IX) because it was so favorable to our contention.

Similar results were obtained with dogs. Three dogs, with marked symptoms of pneumonia, breathed atmospheric air at the average rate of about 5,000 cc. per minute; at 5 per cent of carbon dioxide, they increased the air inspired per minute by 85 per cent. Three normal dogs also breathed about 5,000 cc. per minute; at 5 per cent of carbon dioxide the normal dogs increased the air inspired by 216 per cent. The data for these dogs, three normal and three very ill with pneumonia, are found in Table VI.

TABLE VI.

Three Normal Dogs and Three Very Ill with Pneumonia. The Initial Ventilation Is Practically the Same, but when the Carbon Dioxide in the Inspired Air Rises to 5 Per Cent, the Pneumonic Dogs Give Scarcely One-Third the Normal Reaction.

No. of animal.	Initial ventilation per min.	Percentile increase in cc. breathed per min. as the inspired carbon dioxide increases from 1 to 5 per cent.				
		1	2	3	4	5
	cc.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Normal decerebrate dogs.						
2 A	5,300	14	47	103	181	293
3 B	4,160	10	22	80	131	
16 A	5,360	10	26	63	125	185
16 B	5,320	31	77	137	204	250
16 C	5,800	20	42	69	97	135
Average.....	5,188	17	43	90	148	216
Pneumonic dogs.						
9 D	4,500	15	20	25	33	37
10 B	6,375	11	42	83	94	97
11 A	5,180	19	44	64	94	122
Average.....	5,352	15	35	57	74	85

Additional evidence that the failure of the respiratory mechanism in pneumonia is not due to the encroachment on the respiratory space in the lung is found in the observation that the area of consolidation may remain unchanged while the impairment of the respiratory mechanism increases progressively. The respiratory distress increases while the percussion dullness does not extend.

Moreover, if the failure to react were due to the loss of respiratory space from consolidation of the lung, large consolidations should be accompanied by a large reduction in the carbon dioxide reaction, and small consolidations by small reductions. This is not the case; there is no parallel between the area of consolidation and the amount of the reaction.

Similar evidence may be obtained by injecting starch into the lungs. We have by such injections solidified areas as large as those consolidated in bacterial pneumonia, but in these starch pneumonias

the carbon dioxide reaction was almost normal. The details of these experiments are presented in Table VII.

TABLE VII.

The Reaction to Carbon Dioxide in Dogs in Which Portions of the Lung Were Consolidated by Injecting Starch into the Bronchi.

No. of animal.	Date.	Initial ventilation per min.	Initial respirations per min.	Percentile increase in tidal air per min. as the inspired carbon dioxide increases from 1 to 5 per cent.					Area of consolidation.
				1	2	3	4	5	
	1916	*cc.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
20	Jan. 27	3,008	37	25	53	90	140	220	Right lower and middle lobes.
21	" 31	5,400	45	30	60	89	125	183	Right lower lobe does not expand under air pressure; small part of right middle lobe also consolidated.
23	Feb. 4	3,500	45	20	40	55	117	250	Fully half the lung does not expand when air is blown into trachea.
25	" 5	4,310	72	45	65	110	155	250	Right lower and half of right middle lobes do not distend with strong air blast.
Average....		4,054	49	30	54	86	134	226	

Again, evidence against a mechanical explanation of the observed depression of the respiratory mechanism is afforded by the measurements obtained by Dr. Means from a case of artificial pneumothorax. In this patient, a woman, the x-ray showed a nearly complete collapse of the left lung, from an operation 2 years before this examination. The vital capacity, which had been 2.1 liters before pneumothorax was produced, had fallen to 1.3 liters. The average of two determinations was an increase in total ventilation of 115 per cent when breathing 5 per cent carbon dioxide, and 162 per cent when breathing 6 per cent. A reference to Peabody's[8] results will show that this is normal.

Finally, we call attention to the fact that as the disease advances, the carbon dioxide reaction diminishes. If this were due to an increase in the volume of air breathed per minute at the beginning of each successive test, this initial volume should be greater as the

carbon dioxide reaction falls off, but the contrary is the case; the initial ventilation lessens with the lessening of the reaction. Dog 6 is an example of this observation.

Dog No.	Experiment.	Hrs. before death.	Initial ventilation per min. *cc*	Increase at 3 per cent carbon dioxide. *per cent*
6	A	5	18,630	106
6	B	3	13,800	60
6	C	½	10,400	38

We conclude therefore that our method cannot be impugned on the ground of a lessening of respiratory space in the pneumonic lung.

The Reaction of the Respiratory Mechanism in Pneumonic Cats and Dogs.

TABLE VIII.

The Respiratory Reaction to Carbon Dioxide in Normal Decerebrate Cats.

Cat No.	Experiment.	Date.	Initial ventilation per min.	Initial respirations per min.	Percentile increase in tidal air per min. as the inspired carbon dioxide increases from 1 to 5 per cent.				
					1	2	3	4	5
		1915	*cc.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	A	July 21	485	16	11	55	125		
1	B	" 21	473	17	13	38	115	245	
1	C	" 21	422	17	20	47	112	254	433
1	D	" 21	900	40	20	50	116	174	
2	A	" 22	585	18	13	30	74	160	410
2	B	" 22	289	6	5	10	35	98	206
4	A	Aug. 20	471	7	8	17	25	64	124
4	B	" 20	444	7	2	15	32	53	80
5	A	" 25	741	57	22	46	96	152	205
5	B	" 25	820	82	20	30	53	97	162
5	C	" 25	695	58	32	77	121	173	327
6	A	" 24	657	35	36	64	96	151	260
6	B	" 24	764	33	7	26	55	101	213
6	C	" 25	845	26	13	38			361
7	A	" 24	3 2	18	30	86	124	193	345
7	B	" 24	492	21	20	40	68	134	206
7	C	" 25	256	11	26	64	170	293	370
7	D	" 25	294	14	46	86	136	222	
15	A	Sept. 22	776	37	30	58	112	195	348
15	B	" 22	496	35	27	58	104	180	366
15	C	" 22	319	15	12	37	106	225	253
17		" 23	238	9	24	61	131	248	430
Average.....			539	26	20	47	96	171	283

TABLE IX.

The Respiratory Reaction to Carbon Dioxide in Pneumonic Decerebrate Cats.

Cat No.	Experiment.	Date.	Initial ventilation per min.	Initial respirations per min.	Percentile change in tidal air per min. as the inspired carbon dioxide increases from 1 to 5 per cent.				
					1	2	3	4	5
		1915	*cc.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
10	A	Sept. 7	1,171	78	22	40	54	134	
10	B	" 7	1,767	93	15	27	46		
11	A	" 10	4,300	216	0	6	18		
11	B	" 10	3,000	158	14	26	33	54	
18	A	" 30	1,511	54	14	39	88	134	
18	B	" 30	1,023	46	13	33	98	170	
18	C	" 30	1,025	38	11	44	81	118	112
22		Oct. 13	1,100	55	8	18	30	35	65
23	A	" 13	1,770	59	9	19	29	36	34
23	B	" 13	1,485	55	0	1	7		
23	C	" 13	1,034	36	3	13	31	67	95
26	A	" 16	2,600	65	6	12	25	43	58
26	B	" 16	1,809	67	8	17	26	27	28
26	C	" 16	1,682	40	0	0	6	8	2
Average.....			1,806	76	9	21	41	75	56

The observations on normal and pneumonic cats are given in Tables VIII and IX. They may be summarized as follows:

Carbon dioxide in the inspired air. *per cent*	Increase in volume of tidal air in normal cats. *per cent*	Change in volume of tidal air in pneumonic cats. *per cent*
1	20	9
2	47	21
3	96	41
4	171	75
5	283	56

It is clear that the reaction of the respiratory mechanism to carbon dioxide is greatly diminished.

The greatly impaired reaction of the respiratory mechanism in pneumonia is shown also by a comparison of the normal reaction with (*a*) that of cats moderately ill with pneumonia and (*b*) that of cats near death with this disease. When the carbon dioxide in the inspired air rose to 3 per cent the volume of the tidal air increased:

	per cent.
In normal cats	96
In cats moderately ill with pneumonia	48
" " [9] near death " "	12 / 24

TABLE X.

The Respiratory Reaction to Carbon Dioxide in Normal Decerebrate Dogs.

Dog No.	Experiment.	Date.	Initial ventilation per min.	Initial respirations per min.	Percentile increase in tidal air per min. as the inspired carbon dioxide increases from 1 to 5 per cent.				
					1	2	3	4	5
		1915	*cc.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	A	Nov. 17	3,720	18.6	27	54	96	290	
1	B	" 17	2,828	24.0	12	42	80	120	370
2	A	" 19	5,300	26.5	14	47	103	181	293
3	A	" 22	3,490	36.0	17	34	63	174	305
3	B	" 22	4,160	54.0	10	22	80	131	
16	A	Dec. 14	5,360	25.5	10	26	63	125	185
16	B	" 14	5,320	28.0	31	77	137	204	250
16	C	" 14	5,800	23.2	20	42	69	97	135
17	A	" 15	3,880	36.9	44	96	174	314	457
17	B	" 15	3,590	25.6	4	42	100	181	
Average			4,345	30.0	19	48	96	182	285

TABLE XI.

The Respiratory Reaction to Carbon Dioxide in Pneumonic Dogs.

Dog No.	Experiment.	Date.	Hrs. before death.	Rectal temperature.	Initial ventilation per min.	Initial respirations per min.	Percentile increase in tidal air per min. as the inspired carbon dioxide increases from 1 to 5 per cent.				
							1	2	3	4	5
Temperature above 37.5° C.											
		1915		°C.	*cc.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	A	Sept. 27	5½	38	18,630	162	22	63	106		
9	A	Nov. 29	8	39	5,400	30	24	87	148	178	211
9	B	" 29	4	39	5,400	30	24	83	127	144	152
10	A	" 30	6	39	4,940	77			76	108	153
14	A	Dec. 11	9	39	7,910	72	10	24		104	175
14	B	" 11	6	38.2	11,280	83.5	5	10	17	25	50
14	C	" 11	4½	38	10,090	84	4	8	12	13	19
Average			6	38.6	9,093	77	15	46	81	95	127

[9] This group includes two cats that died at the close of the observation. If these are excluded, the increase becomes 24 per cent instead of 12 per cent.

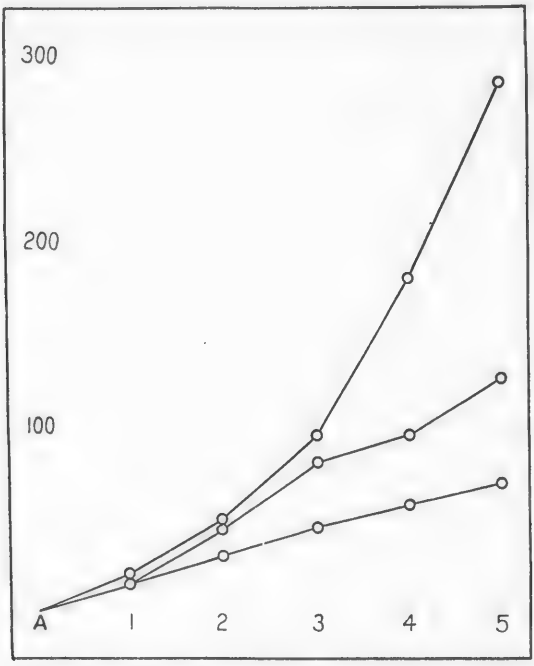
TABLE XI—*Concluded.*

Dog No.	Experiment.	Date.	Hrs. before death.	Rectal temperature.	Initial ventilation per min.	Initial respirations per min.	Percentile increase in tidal air per min. as the inspired carbon dioxide increases from 1 to 5 per cent.				
							1	2	3	4	5
Temperature 37.5° C. and below.											
		1915		°C.	*cc.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	B	Sept. 27	3½	35	13,800	138	8	28	60		
6	C	" 27	½	33.5	10,400	130	18	34	38		
9	C	Nov. 29	2	37	6,720	160	31	57	70	72	74
9	D	" 29	¾	36	4,500	150	15	20	25	33	37
10	B	" 30	2	37	6,375	85	11	42	83	94	97
11	A	" 29	1	34	5,180	115	19	44	64	94	122
14	D	Dec. 11	3	37	10,320	76.5	2	4	12	20	25
14	E	" 11	1½	35	9,590	68.5	6	12	17	39	50
Average			1¾	35.6	8,361	115	14	30	46	59	68

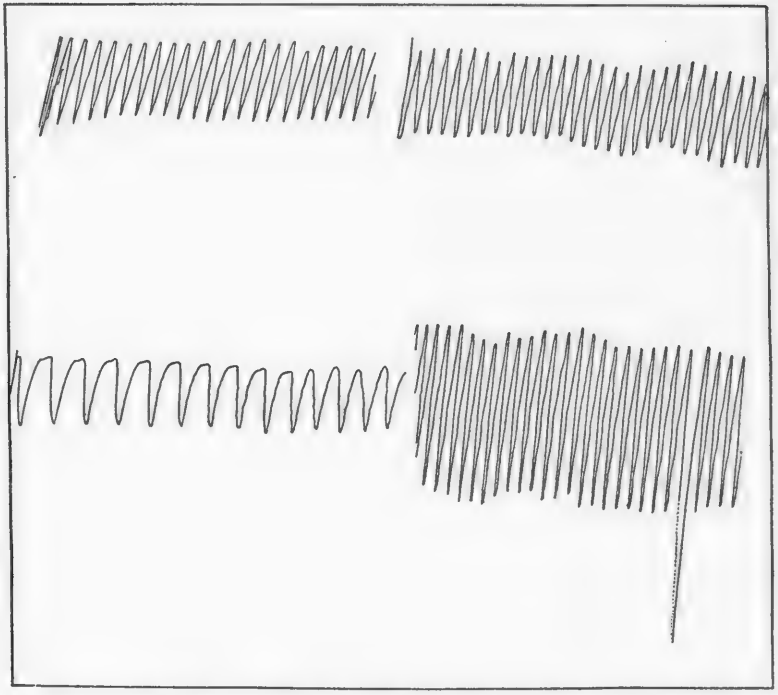
The observations upon dogs are given in Tables X and XI. They may be summarized as follows:

Carbon dioxide in the inspired air. *per cent*	Increase in volume of tidal air in normal dogs. *per cent*	Increase in volume of tidal air in pneumonic dogs. Temperature above 37.5°C. *per cent*	Temperature 37.5°C. or less. *per cent*
1	19	15	14
2	48	46	30
3	96	81	46
4	182	95	59
	285	127	68

These observations are shown graphically in Text-fig. 4. Text-fig. 5 reproduces the spirometer curves of a normal and a pneumonic dog.



TEXT-FIG. 4. The increase in tidal air in normal decerebrate dogs (uppermost curve), in pneumonic dogs with temperature above 37.5° C. (middle curve), and in pneumonic dogs with temperature 37.5° C. and below (lowest curve). The numbers at the left give the percentile increase in tidal air; the numbers below give the per cent of carbon dioxide. The data for these curves are the averages in Tables X and XI.



TEXT-FIG. 5. A comparison of the pneumonic and the normal respiratory reactions to carbon dioxide. The two upper curves show the tidal air at 1 and 5 per cent of carbon dioxide in Dog 14, Dec. 11, 1915, 5 hours before death from pneumonia. The two lower curves show the tidal air at 1 and 5 per cent of carbon dioxide in a normal decerebrate dog, Dec. 15, 1915.

It will be noted that the pneumonic dogs are divided into those moderately ill (rectal temperature above 37.5°C.) and those gravely ill (temperature 37.5°C. or less). The result of the disease is clearly seen by a comparison of the reactions of these groups when the inspired air contained 5 per cent of carbon dioxide.

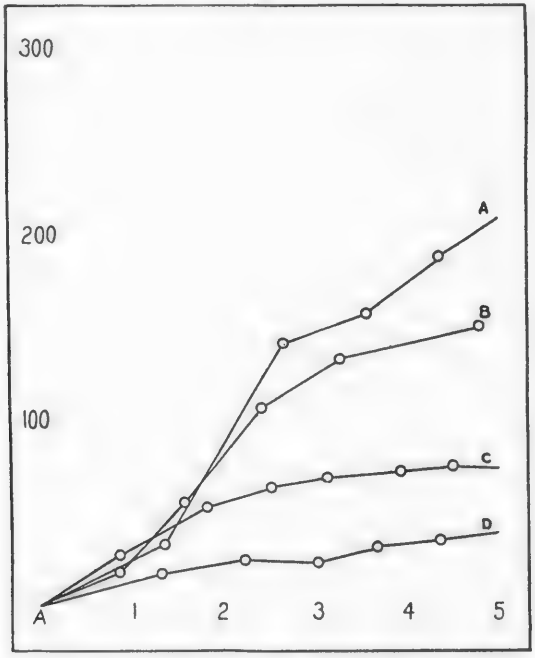
Reaction at 5 per cent of carbon dioxide:

	per cent
In normal dogs	285
In dogs moderately ill with pneumonia	127
" " gravely ill with pneumonia	68

The progressive loss of the respiratory reaction in pneumonia is well shown by the observations made on Dog 9, 8, 4, 2, and ¾ hours before death.

	Percentile increase in tidal air of Dog 9 as the carbon dioxide in the inspired air rose from 1 to 5 per cent.				
Hrs. before death.	1 *per cent*	2 *per cent*	3 *per cent*	4 *per cent*	5 *per cent*
8	24	87	148	178	211
4	24	83	127	144	152
2	31	57	70	72	74
¾	15	20	25	33	37

These observations are shown graphically in Text-fig. 6.



TEXT-FIG. 6. The reaction to carbon dioxide in Dog 9, Nov. 29, 1915, 8, 4, 2, and ¾ hours before death from pneumonia. The numbers at the left give the percentile increase in tidal air as the carbon dioxide inspired increases; the numbers below indicate the per cent of carbon dioxide. It is clear that the respiratory mechanism fails steadily.

The pneumonias thus far presented have been produced with *Bacillus pneumoniæ*, which is not often seen in clinical practice. The

ordinary pneumonia is caused by the pneumococcus. We find, however, no essential difference in the action of this organism—the reaction of the respiratory mechanism to carbon dioxide is lessened and finally abolished with the pneumococcus as with the bacillus of Friedländer. The following experiment is instructive.

On Apr. 12, 1916, a dog weighing 13.5 kilos received in the right bronchus 58 cc. of a liquid culture[10] of the pneumococcus. On Apr. 13 the rectal temperature at 9.30 a.m. was 39° C. The dog was tracheotomized at this time. At 9.45 a.m. the first carbon dioxide reaction was taken; the dog was able to walk about. At 3 p.m. the temperature was 39.9° C.; the dog could not maintain the sitting position; the second carbon dioxide reaction was measured. At 5 p.m. the temperature was 39.7° C.; the dog was semicomatose; the third carbon dioxide reaction was measured. At 6.20 p.m. the temperature was 39.7° C.; deep coma; corneal reflex present; hind legs becoming stiff; head slightly retracted; carbon dioxide reaction absent. At 6.30 p.m. the dog died. The autopsy showed typical red hepatization of the whole right lower, one-half the right middle, and one-half the right upper lobe.

The measurements in this dog were as follows:

	Percentile change in cc. breathed per min. as the carbon dioxide in the inspired air rose from 1 to 5 per cent.				
Hrs. before death.	1 *per cent*	2 *per cent*	3 *per cent*	4 *per cent*	5 *per cent*
$8\frac{3}{4}$	13	28	43	57	114
$3\frac{1}{2}$	7	18	40	100	171
$1\frac{1}{2}$	No reaction.			5	27
$\frac{1}{6}$	" "				

Although the course of the temperature differs in this animal from that usually observed with Friedländer's bacillus, there is no difference in the failure of the respiratory mechanism.[11]

CONCLUSIONS.

1. The reaction of the respiratory mechanism to carbon dioxide is greatly diminished in pneumonia.
2. The graver the disease, the less the reaction.

[10] The culture medium is glucose broth to which calcium carbonate has been added.

[11] We take pleasure in acknowledging our indebtedness to the industry and devotion of Mr. Luigi Freni, nine years an assistant in this laboratory.

THE INFLUENCE OF THE VAGUS NERVES UPON CONDUCTION BETWEEN AURICLES AND VENTRICLES IN THE DOG DURING AURICULAR FIBRILLATION.

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PLATE 43.

(Received for publication, July 5, 1916.)

The normal heart beat of the dog is affected differently by right and left vagus stimulation. Right vagus stimulation causes inhibition of impulse formation, slowing the rate of the heart beat or causing complete standstill. Left vagus stimulation has a less marked effect upon impulse formation, but seems to inhibit the conduction of impulses from auricles to ventricles more than right vagus stimulation. This difference was pointed out by Cohn,[1] who recorded the cardiac action by means of the string galvanometer. The writer[2] confirmed these observations in a series of experiments in which the same technique was employed. Although in these series of experiments it seemed clear that conduction between the auricles and ventricles was distinctly influenced more by the action of the left vagus, the comparison of the two nerves in this respect was confused by the predominant action of the right vagus in causing auricular slowing or stoppage. This difficulty has been pointed out by Cohn and Lewis,[3] who investigated the effect of the two nerves on conduction when auricular rate was maintained at a constant level by rhythmic induction shocks, at, or a little above threshold value, applied to the right auricle. They found that under these conditions profound

[1] Cohn, A. E., On the Differences in the Effects of Stimulation of the Two Vagus Nerves on Rate and Conduction of the Dog's Heart, *J. Exp. Med.*, 1912, xvi, 732.

[2] Robinson, G. C., The Influence of the Vagus Nerves on the Faradized Auricles in the Dog's Heart, *J. Exp. Med.*, 1913, xvii, 429.

[3] Cohn, A. E., and Lewis, T., The Predominant Influence of the Left Vagus Nerve upon Conduction between the Auricles and Ventricles in the Dog, *J. Exp. Med.*, 1913, xviii, 739.

changes in conduction were obtained, which were generally more marked when the stimulus was applied to the left vagus than to the right.

The experiments to be reported in this paper are in some ways comparable to those of Cohn and Lewis, but instead of auricular impulses being maintained by rhythmic induction shocks, they were maintained by throwing the auricles into a state of constant activity by faradization. The result also is somewhat different. The type of auricular activity which results from faradization of the auricles is not true fibrillation alone, but consists in the presence of true fibrillary activity combined with auricular tachycardia involving large masses of the musculature.[2] For the purpose of the present paper, however, it seems unessential to draw a distinction between these two types of activity, and both will be spoken of as fibrillation.

The technique of the experiments has been described in a previous paper,[2] where the results of the effect of the stimulation of each nerve on the faradized auricles were reported, and will be referred to again only briefly. The electrocardiographic curves obtained in this series of experiments have been reviewed, especial attention being paid to two points. The first point is the relative effect of stimulation of the two nerves on conduction during auricular fibrillation. The second is the relative effect of left vagus stimulation in inhibiting the conduction of impulses set up by the normally beating auricles and in inhibiting the conduction of impulses set up by the fibrillating auricles. This comparison is possible in those experiments in which the normally beating auricles continued to beat during left vagus stimulation. By comparing the electrocardiographic records which showed this condition with those obtained during left vagus stimulation from the same animal while the auricles were fibrillating, it is possible to conclude whether impulses from the fibrillating auricles are more or less easily blocked by left vagus activity than normal auricular impulses.

No such comparison is possible with the right vagus, as a condition of auriculoventricular block was practically never produced in the normally beating heart with right vagus stimulation, auricular stoppage being a constant result.

EXPERIMENTAL.

In the series of twenty-seven experiments, thirteen allow a comparison of the effect of the two nerves on conduction during auricular fibrillation. Those experiments in which there was ventricular escape with auricular stoppage during the normal cardiac mechanism were discarded, for if ventricular escape should occur during vagus stimulation when the auricles were fibrillating, a false idea of the effect of vagus activity on conduction might be given.

Nine experiments allow a comparison of the effect of left vagus stimulation on conduction during the normal cardiac mechanism and during auricular fibrillation. In these experiments the auricular activity persisted when the left vagus was stimulated during the normal cardiac mechanism, and ventricular escape did not occur.

The experiments were carried on with the dogs under ether anesthesia. Electrodes were applied to the right fore leg and the left hind leg, and records were made with the Edelmann string galvanometer. The chest was opened and two clips to which stimulating wires were attached, were applied to the right auricle through a small opening in the pericardium. Each vagus was dissected out, and shield electrodes were applied. The auricles were faradized with varying strengths of currents, but the vagi were stimulated with a faradic current of constant strength, the secondary coil being usually pulled out 5 cm. In a few experiments a slightly weaker current was used. In some animals prolonged auricular fibrillation was produced by a single faradization, while in others frequent periods of faradization were necessary. This difference had no apparent effect on the action of the two nerves. The results of the experiments are given in tabular form, the details of all records which bear on the two points considered being shown by certain abbreviations.

In the first column is given the curve number, and in the second the type of auricular activity, N. B. signifying normal beat and A. F. auricular fibrillation. In the third column the duration of vagus stimulation in seconds is given, and in the fourth and fifth the effects of stimulation of the left and right nerves respectively are given. In describing the effects of vagus stimulation the term stoppage signifies arrest of the ventricles during auricular fibrillation, or of the whole heart during the normal beat. Complete block means that the auricles continued without ventricular responses; 2 : 1, 3 : 1, etc. express the ratio of auric-

ular and ventricular contractions, and the degree of partial heart block; $2:1^3$ indicates the occurrence of three 2 : 1 cycles; P−R+ signifies a prolongation of the conduction interval. Figures in brackets indicate the length of time in seconds an effect has persisted after the termination of vagus stimulation. Other abbreviations have meanings that are self-evident.

The details of each experiment are given, followed by a tabulated summary of the results of all the experiments (Table I).

EXPERIMENT I.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
11	A. F.	4.2	Slowing, V. rate 102.	
15 A	" "	2.2		Stoppage, 2.6 sec. (0.4).
15 B	" "	2.4		" 3.0 " (0.6).
16	" "	4.3		" 4.4 " (0.1).

Summary.—Vagus action is uncontrolled on the normal beat. The right vagus is markedly more effectual in blocking impulses from the fibrillating auricles than the left vagus. The experiment is not conclusive, as there was but one left vagus stimulation.

EXPERIMENT II.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
9	A. F.	4.6		Stoppage, 1 beat in 5.0 sec.
14	" "	4.5	Stoppage, 2 beats in 6.2 sec.	
15	" "	5.0		" 2 beats " 5.0 "
16	" "	5.0	" 1 beat " 5.0 "	
18	" "	5.0	" 1 " " 7.6 "	
19	" "	5.0		" 1 beat " 6.2 "

Summary.—Vagus stimulation is uncontrolled on the normal beat. The right and left vagi are equally effectual in blocking impulses from the fibrillating auricles.

EXPERIMENT III.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
8	A. F.	5.6	Stoppage, 5.9 sec. (0.3). (Fig. 1).	
9	N. B.	4.6	Complete block, 4.6 sec. (0). A. rate, 110.5 (Fig. 2).	
10	A. F.	2.8		Stoppage, 3.2 sec. (0.4).
11	N. B.	3.9		" 3.9 " (0).

Summary.—The usual differences between stimulation of the right and left vagi are shown. The right and left vagi are equally effectual in blocking impulses from the fibrillating auricles. The left vagus stimulation caused blocking of impulses from the normally beating auricles as effectually as impulses from the fibrillating auricles.

EXPERIMENT IV.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
4	N. B.	5.4	P−R+ slight slowing.	
5	" "	5.5		Marked slowing, 3 beats in 5.6 sec.
8	A. F.	6.0	Slowing, 6 beats in 5.8 sec.	
10	N. B.	5.8	2 : 1^1, P−R+ slight slowing. Rate 120.	
11	" "	5.6		P−R slightly +, slowing.
14	A. F.	5.6		Slowing, 8 beats in 5.0 sec.
15	" "	6.0	Marked slowing, 2 beats in 3.6 sec.	
16	" "	6.4		Slight slowing, 14 beats in 5.6 sec.
17	" "	6.2	Marked slowing, 3 beats in 4.6 sec.	

Summary.—The usual differences between stimulation of the right and left vagi are shown, but they are not striking. The left vagus is more effectual than the right in blocking impulses from the fibrillating auricles, and is more effectual in blocking impulses from the fibrillating auricles than from the normally beating auricles.

EXPERIMENT V.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
12	N. B.	6.6	Complete block, 1.4 sec. P—R+. A. slight slowing.	
13	" "	6.4		Stoppage, 2.2 sec. Ectopic ventricular beats. A. much slowed.
16	A. F.	4.8	Stoppage, 1.9 sec. Slight slowing, 2.6 sec.	
17	" "	5.8		Stoppage, 5.6 sec. 4 ectopic ventricular beats.
18	" "	6.0		Stoppage, 5.6 sec. 4 ectopic ventricular beats.
19	" "	5.2	Stoppage, 2.1 sec. Slight slowing, 3.2 sec.	
20	" "	8.8	Stoppage, 2.2 sec. Slowing, 2.0 sec.	
21	" "	7.2		Stoppage, 6.8 sec. 5 ectopic ventricular beats.

Summary.—The effect of stimulation of the right and left vagus on the normally beating heart is confused by ectopic ventricular impulses. The right vagus is more effectual in blocking impulses from the fibrillating auricles than the left. The left vagus is slightly more effectual in blocking impulses from the fibrillating auricles than from the normally beating auricles.

EXPERIMENT VI.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
7	A. F.	5.7		Stoppage, 7.8 sec. (2.1).
8	" "	6.4	Stoppage, 8.8 sec. (2.4).	
9	" "	7.6		" 10.4 " (2.8).
10	" "	8.7	" 10.8 " (2.1).	
12	N. B.	4.0		" 5.2 " (1.2). P—R+ . 2 beats.
13	" "	5.0	Complete block, 6.2 sec. (1.2). A. rate 92.	
14	A. F.	6.6	Stoppage, 7.4 sec. (0.8).	
15	" "	5.0		Stoppage, 7.0 sec. (2.0).
16	N. B.	3.7	Complete block, 4.5 sec. (0.8). A. rate 108.	
17	" "	5.0		" 6.2 " (1.2).
18	A. F.	5.8		" 8.0 " (2.2).
19	" "	6.9	Stoppage, 9.6 sec. (2.7).	

Summary.—The usual differences between stimulation of the right and left vagi are strikingly shown. The right and left vagi are equally effectual in blocking impulses from fibrillating auricles. The left vagus is as effectual in blocking the impulses from the normally beating auricles as those from the fibrillating auricles.

EXPERIMENT VII.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
5	A. F.	3.8		Stoppage, 6.0 sec. (2.2).
7	" "	5.2	Stoppage, 9.0 sec. (3.8).	
9	" "	6.8		" 9.4 " (2.6).
10	" "	6.0	" 9.0 " (3.0?).	
11	N. B.	6.7		" 9.2 " (2.5).
15	" "	3.0	" 5.0 " (2.0) $2:1^3$.	
16	" "	11.4		" 14.2 " (2.8).
17	" "	7.6	Stoppage, 8.6 sec. (1.0) $3:1^1$, $2:1^3$.	
18	A. F.	5.0	Stoppage, 7.6 sec. (2.6).	

Summary.—The usual differences between stimulation of the right and left vagi are shown, but they are not striking. The left vagus is slightly more effec-

tual than the right in blocking impulses from the fibrillating auricles, ventricular stoppage outlasting the period of left vagus stimulation longer than stimulation of the right. The effect of left vagus stimulation on the blocking of impulses from the normally beating auricles cannot be ascertained on account of stoppage of the auricles, which it caused.

EXPERIMENT VIII.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
5	A. F.	4.8	Marked slowing, V. rate 53.	
12	" "	5.4		Marked slowing, V. rate 73.

Summary.—A comparison of the effect of stimulation of the two nerves on the normally beating heart was not obtained. The left nerve is more effectual in blocking impulses from the fibrillating auricles than the right.

EXPERIMENT IX.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
6	N. B.	1.8		Stoppage, 2.2 sec. (0.4).
7	" "	3.8	Complete block, 4.0 sec. (0.2). A. rate 120.	
9	" "	3.2		Stoppage, 2.2 sec.; then slowing. P—R slightly+.
10	" "	4.0	$3:1^1, 2:1^3$.	
13	" "	6.6	$6:1^1, 2:1^5$.	
14	" "	3.2		Stoppage, 2.2 sec.; then slowing. P—R not +.
16	A. F.	4.4	Stoppage, 2.2 sec.; then marked slowing. Rate 45.	
17	" "	7.8		Stoppage, 0.8 sec.; then slowing. Rate 123.
18	" "	6.0	Stoppage, 3.3 sec. 2 beats in 6.2 sec.	
20	" "	5.8		Slowing, 6 beats in 5.6 sec.

Summary.—The usual differences between stimulation of the right and left vagi are strikingly shown. The left vagus is more effectual in blocking impulses from the fibrillating auricles than the right, while it causes blocking of impulses

from the normally beating auricles and from fibrillating auricles in an equal degree.

Curves 13, 14, 16, and 17 have been reproduced in a previous paper[2] as figures 4, 3, 6, and 5 respectively.

EXPERIMENT X.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.*	Right vagus effects.
		sec.		
4	N. B.	3.0		Stoppage, 3.0 sec. (0) $2:1^1$.
5	" "	5.1	$3:1^1$, $2:1^3$. P−R+. A. rate 103.	
8	A. F.	4.6		" 5.6 sec. (1.0).
10	" "	6.1	Stoppage, 3.0 sec. 3 beats in 6.6 sec.	
11	" "	5.4		" 5.4 " (0).
14	N. B.	6.2	$2:1^5$. P−R+. A. rate 58.	
15	" "	3.0		" 3.0 " (0). $2:1^1$, P−R+.
20	" "	4.1	Stoppage, 3.6 sec. $5:1^1$, $2:1^1$. A. rate 80.	
21	A. F.	3.0	Stoppage, 3.2 sec. (0.2).	

* Nerves stimulated with secondary coil out 6 cm.

Summary.—The usual differences between stimulation of the right and left vagi are strikingly shown. Right and left vagi are equally effectual in blocking impulses from the fibrillating auricles. The left vagus is as effectual in blocking impulses from the normally beating auricles as those from the fibrillating auricles.

EXPERIMENT XI.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.*	Right vagus effects.
		sec.		
4	N. B	4.2		Stoppage, 6.4 sec. (1.8).
5	" "	4.8	Complete block, 5.2 sec. (0.4). A. rate 116.	
6	" "	2.3		" 4.0 " (1.7).
7	" "	3.7	Complete block, 4.4 sec. (0.7). A. rate 100.	
8	A. F.	4.0		" 4.4 " (0.4).
9	" "	5.1	Stoppage, 4.8 sec.	
11	" "	6.3	" 6.6 " (0.3).	
20	N. B.	5.4	Complete block, 3.0 sec. A. rate 117.	
21	" "	1.4		" 4.4 " (3.0).
22	A. F.	4.4	Stoppage, 4.2 sec.	
23	" "	4.9		" 6.2 " (1.7).

* Nerves stimulated with secondary coil out 7 cm.

Summary.—The usual differences between stimulation of the right and left vagi are strikingly shown. The right vagus is slightly more effectual in blocking impulses from the fibrillating auricles than the left, as shown by the fact that ventricular stoppage always outlasts the periods of right vagus stimulation, while it usually does not outlast the periods of left vagus stimulation. The left vagus is as effectual in blocking the impulses from the normally beating auricles as those from the fibrillating auricles.

EXPERIMENT XII.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
9	A. F.	4.8	Marked slowing, 6 beats in 4.8 sec.	
10	" "	5.4		Slight slowing.
11	N. B.	5.6	$2:1^3$. P−R++. 3 V. beats in 2.6 sec.	
12	" "	7.0		No effect.
13	" "	4.4	$2:1^3$. P−R++. 3 V. beats in 2.6 sec.	
14	" "	8.4		" "
17	" "	4.2		Stoppage, 1.6 sec.; then marked slowing.
18	" "	5.2	$2:1^5$. P−R++. 6 V. beats in 4.4 sec.	
26	A. F.	6.6		No effect.

Summary.—The right vagus is not effectual. The left vagus is as effectual in blocking impulses from the normally beating auricles as those from the fibrillating auricles.

EXPERIMENT XIII.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
3	N. B.	5.0		Stoppage, 4.0 sec. P−R+.
4	" "	5.0	Stoppage, 4.6 sec. 1 blocked A. beat, P−R+.	
5	" "	3.2		" 3.6 " (0.4). P−R+.
6	" "	3.4	Stoppage, 3.2 sec. 1 blocked A. beat, P−R+.	
9	A. F.	5.0		Stoppage, 5.6 sec. (0.6).
10	" "	5.4	Stoppage, 5.6 sec. (0.2).	
11	" "	5.0		" 6.0 " (1.0).
12	" "	6.2	" 6.0 "	
13	N. B.	5.2		" 5.6 " (0.4). 2 blocked A. beats. P−R+.
14	" "	5.4	" 5.4 " P−R+.	
16	" "	4.0		Stoppage, 5.6 sec. (1.6). P−R not +.
17	" "	4.0	" 4.2 " 1 blocked A. beat.	
20	A. F.	5.6		Stoppage, 6.6 sec. (1.0).
22	" "	3.2	Stoppage, 3.2 sec.	
23	" "	5.0		" 5.8 " (0.8).

Summary.—The usual differences between stimulation of the right and left vagi are shown, but they are only slight. The right vagus is slightly more effectual in blocking impulses from the fibrillating auricles than the left, as is indicated by the fact that blocking always outlasts the periods of right vagus stimulation, while it usually does not outlast the periods of left vagus stimulation. The effect of left vagus stimulation on blocking of impulses from the normally beating auricles cannot be ascertained on account of the stoppage of auricles which it causes.

EXPERIMENT XIV.

Curve No.	Auricular activity.	Duration of vagus stimulation.	Left vagus effects.	Right vagus effects.
		sec.		
3	N. B.	3.6		Stoppage, 4.0 sec. (0.4).
4	" "	7.8	$3:1^1, 2:1^8$. 9 V. beats in 7 sec. A. rate 165. V. rate 80.	
7	A. F.	4.3		" 3.8 "
8	" "	5.4	Slowing. 7 beats in 4.2 sec. V. rate 109.	

Summary.—The usual differences between stimulation of the right and left vagi are strikingly shown. The right vagus is strikingly more effectual than the left in blocking impulses from the fibrillating auricles. The left vagus is nearly or entirely as effectual in blocking impulses from the fibrillating auricles as those from the normally beating auricles.

The results of these experiments are given in tabular form (Table I). In the third column are given the effects of stimulation of the right and left vagi on the normally beating heart. These effects serve as a control for the activity of the nerves during auricular fibrillation, except in three experiments in which the auricles were always faradized before the vagi were stimulated. When the effects are described as "usual," it signifies that the right nerve was more effectual in causing stoppage of the whole heart, while the left nerve was apparently more effectual in causing blockage of the impulses from the auricles.

The effect that stimulation of each nerve had on conduction during auricular fibrillation has been estimated by the degree of ventricular slowing which took place or the length of time ventricular stoppage occurred relative to the duration of vagus stimulation. These results are given in the fourth double column. In the last double column are given the effects which left vagus stimulation had on the ventricular rate during auricular fibrillation and during the normal beat in those experiments in which normal auricular activity persisted during left vagus stimulation.

TABLE I.

Tabulated Results of Experiments.

Experiment No.	Series No.	Effects on normal beats.	Effect on conduction during A.F.		Effect of left nerve on conduction.	
			Right.	Left.	A.F.	N.B.
I	103	Not recorded.	+	−	Not determined.	
II	105	" "	Equal.	Equal.	" "	
III	108	Usual, marked differences.	"	"	Equal.	Equal.
IV	111	" slight "	−	+	+	−
V	112	Undetermined.	+	−	+	−
VI	113	Usual, marked differences.	Equal.	Equal.	Equal.	Equal.
VII	114	" slight "	−	+ slight.	Not determined.	
VIII	115	Not recorded.	−	+	" "	
IX	118	Usual, marked differences.	−	+	Equal.	Equal.
X	121	" moderate "	Equal.	Equal.	"	"
XI	122	" marked "	+ slight.	−	"	"
XII	125	Right nerve ineffectual.	Not determined.		"	"
XIII	126	Usual, slight differences.	+ slight.	−	Not determined.	
XIV	127	" marked "	+	−	Equal.	Equal.
Totals 14.		9 characteristic. 5 undetermined.	5+	4+ 4 equal. 1 undetermined.	2+	0+ 7 equal. 5 undetermined.

DISCUSSION.

The thirteen experiments in which the effect of right and left vagus stimulation on ventricular activity during auricular fibrillation was compared indicate that there is no constant difference. The right nerve was more effectual in slowing or stopping the ventricles in five experiments, the left nerve was more effectual in four, while in four experiments the two nerves were equally effectual. Impulses apparently continue to be sent down frequently toward the ventricles. The fact that ventricular activity is strikingly inhibited by vagus stimulation under either condition indicates, therefore, that the impulses from the auricles are blocked. The degree to which the ventricular activity is diminished or the duration of ventricular stoppage relative to the duration of vagus stimulation may be taken as the measure of the effect of vagus stimulation on conduction between auricles and ventricles.

The experiments fail to show any constant difference in the effect which stimulation of the right and of the left vagus nerves has on conduction between auricles and ventricles of the dog's heart when auricular fibrillation is present. These results are not in accord with those of Cohn and Lewis, who found a difference in the influence of the two nerves on auriculoventricular conduction when the auricular activity was maintained during vagus stimulation by rhythmic electric stimulation. It is possible that this disagreement is to be found in the difference in auricular activity. In a previous paper[2] it was pointed out that the auricular activity following faradization is differently altered by stimulation of the two nerves. Stimulation of the right nerve causes a type of auricular activity in which the separate muscle fibers become more independently active, while stimulation of the left nerve causes the auricular activity to be of a coarser type, more nearly approaching auricular tachycardia.

The difference in auricular activity does not seem to be sufficient to explain the differences between the results of the experiments reported here and those of Cohn and Lewis. The reason for the discrepancy is obscure.

The nine experiments which afford an opportunity for comparing the effect of left vagus stimulation on conduction during the normal beat and during auricular fibrillation show that the type of auricular activity has apparently no influence on the effect which stimulation of the left vagus has on auriculoventricular conduction. With two exceptions the ventricular stoppage or slowing caused by the stimulation when the auricles were fibrillating was identical with that obtained during the normal heart beat. In only one experiment was left vagus stimulation definitely more effectual in blocking impulses from the fibrillating auricles than from the normally beating auricles. These results indicate that the type of auricular activity has no influence on the degree to which impulses are blocked by vagus stimulation. This fact is of interest as bearing upon the action of digitalis in auricular fibrillation. It has been suggested that digitalis is especially potent in blocking impulses sent to the ventricles from fibrillating auricles. In the light of these experiments it would seem that the character of the auricular activity whether coordinate or fibrillatory plays no part in the effectiveness of the drug.

CONCLUSIONS.

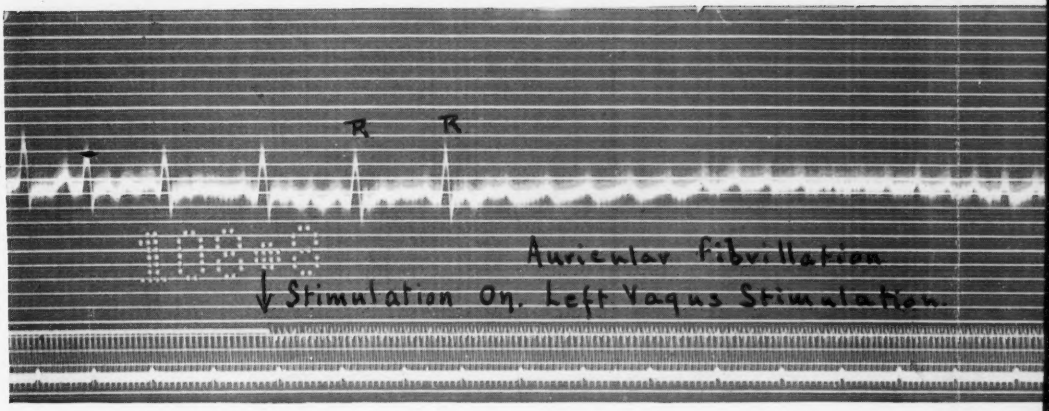
The experiments that have been reported indicate that stimulation of either the right vagus or the left vagus nerve is equally effectual in blocking impulses from the auricles to the ventricles when auricular fibrillation is present. Stimulation of the left vagus nerve is as effectual in blocking impulses from the normally beating auricles as from the auricles when in a state of fibrillation, and the type of auricular activity has apparently no influence on the effect which stimulation of the left vagus has on auriculoventricular conduction.

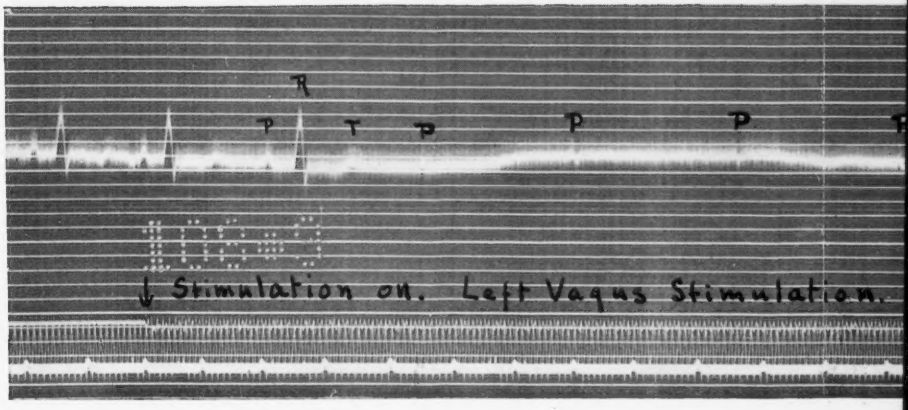
EXPLANATION OF PLATES.

PLATE 43.

FIG. 1. Experiment III. The effect of left vagus stimulation during auricular fibrillation. Ventricular stoppage for 5.9 seconds. Stimulation of 5.6 seconds' duration.

FIG. 2. Experiment III. The effect of left vagus stimulation during normal auricular activity. Ventricular stoppage for 4.6 seconds. Stimulation of 4.6 seconds' duration.


R R
Auricular fibrillation.
↓ Stimulation On. Left Vagus Stimulation.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↓ Stimulation on. Left Vagus Stimulation.

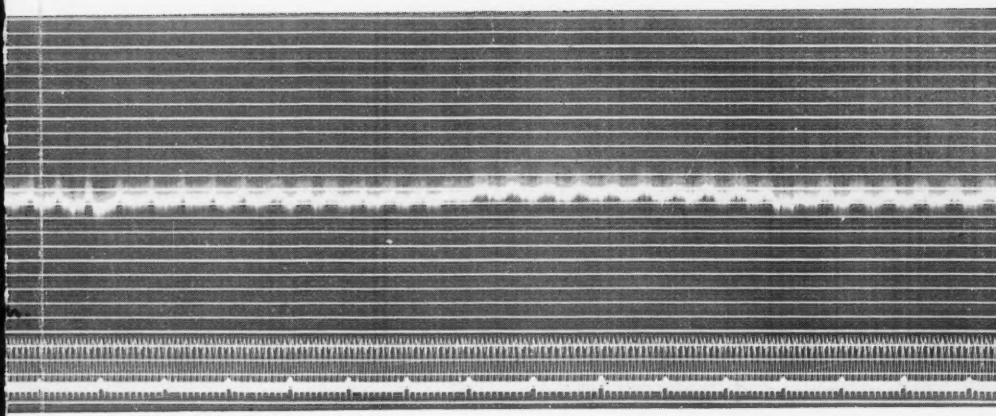


FIG. 1.

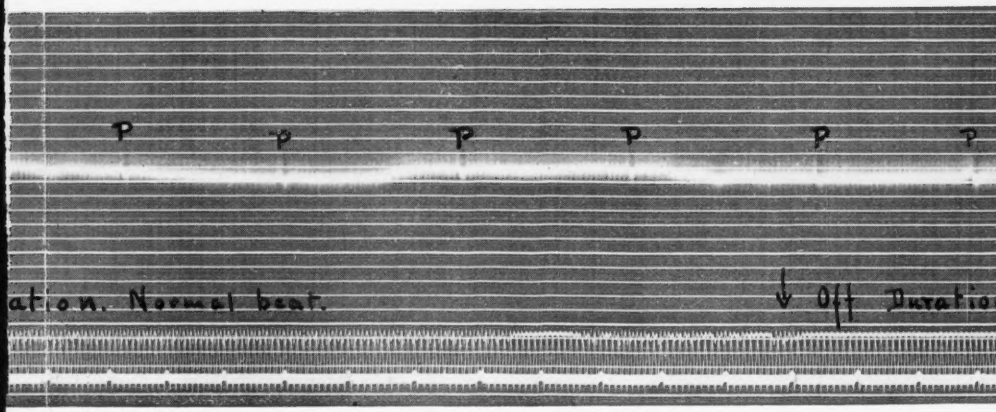
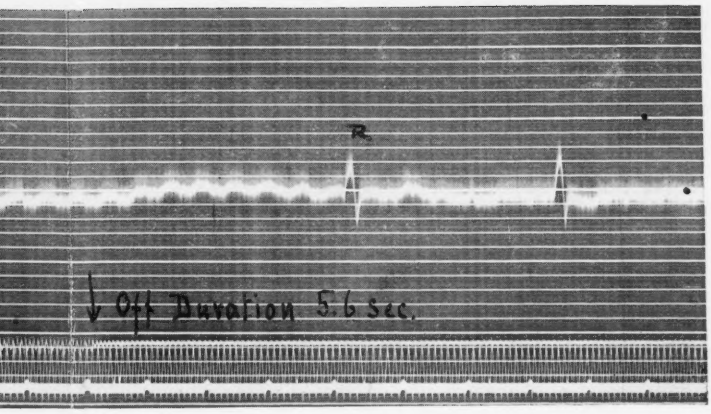
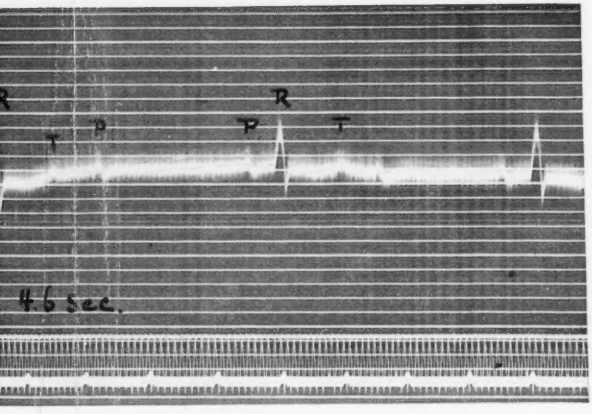


FIG. 2.





(Robinson: Conduction between Auricles and Ventricles.)